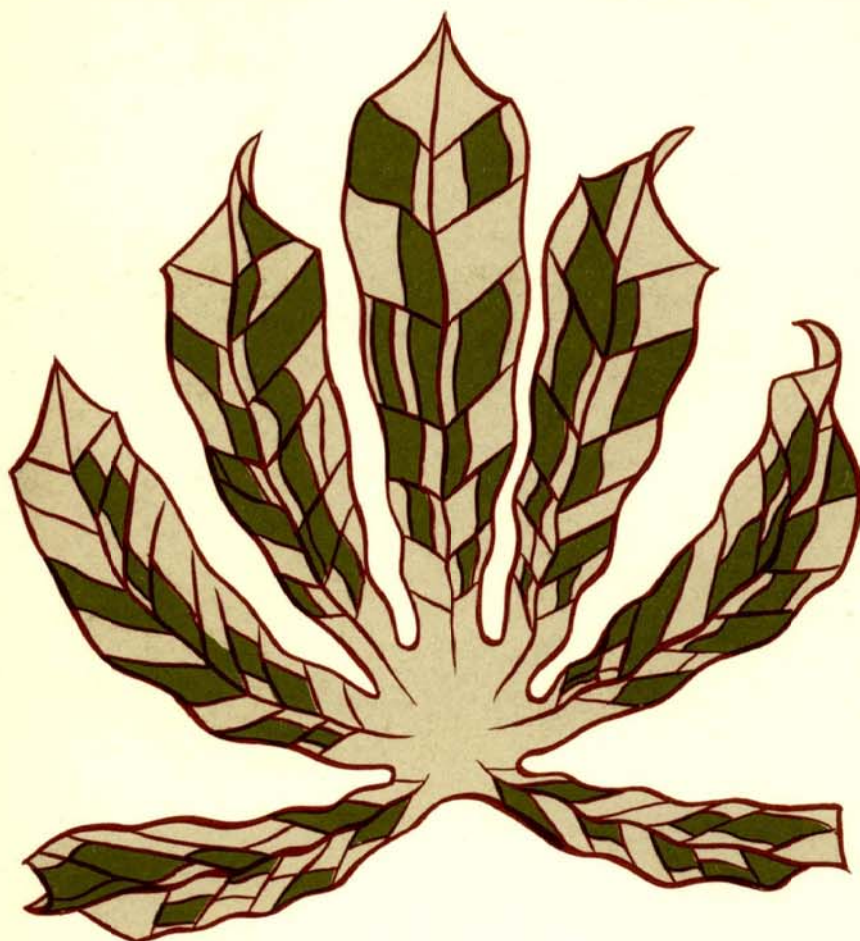


African Cassava MOSAIC



Report of an Interdisciplinary Workshop held at Muguga, Kenya, 19-22 February 1976

Editor: Barry L. Nestel

Cosponsored by the East African Agriculture and Forestry Research Organization
and the International Development Research Centre

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Editor: **Barry L. Nestel**
International Development Research Centre
Bogota, Colombia

Cosponsored by the
East African Agriculture and Forestry Research Organization
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Introduction

This is the tenth publication in the International Development Research Centre (IDRC) series of cassava reports. It is a report on a workshop held at the Muguga headquarters of the East African Agriculture and Forestry Research Organization (EAAFRO) on 19–22 February 1976. The meeting was cosponsored by EAAFRO and IDRC and was the second workshop on cassava mosaic. The earlier workshop dealing with virus and mycoplasma-like diseases of cassava was held at the International Institute of Tropical Agriculture (IITA) in December 1972. The present workshop had the objective of reviewing the present state of knowledge and the current status of research relating specifically to African cassava mosaic (CMD). It also sought to identify future priorities for research in this field.

The workshop was opened by *Dr E. Wangati*, the Deputy Director of EAAFRO, who stressed the role that cassava played in the agricultural economy of East Africa and drew attention to the significant research on cassava mosaic which had started in 1937 and had continued up to the present time. Some of the key scientists associated with this work had been part of the EAAFRO organization throughout much of this period.

The role of cassava in the Kenyan agricultural economy was discussed in the first paper by *Seif and Chogoo* who described the present status of research. They felt that the two major problems inhibiting the expansion of cassava production were mosaic disease and marketing. Mosaic could be largely controlled by cultural practices but there was no incentive to the farmer to introduce new practices for a crop that was grown mainly for subsistence, until a sounder marketing structure evolved and, possibly, a processing industry developed.

The second paper by *Bock and Guthrie* of EAAFRO described their recent work which suggested that African Cassava Mosaic on the Kenya coast was associated with two virus particles. Bock and Guthrie established and maintained a collection of cassava showing tolerance to mosaic. This was largely obtained from material derived from the lengthy period of cross-breeding work initiated by *Storey* nearly 40 years ago. The EAAFRO workers are studying the epidemiology of the disease and suggested that in the coastal areas the spread of the disease was largely caused by man and that through improved cultural practices it was possible to keep the disease at a low incidence in the field. Their work on identifying the causative organism suggested that the disease involved two serologically related but distinct viruses at least one of which was a doublet which produced the symptoms originally described as “brown streak” disease.

At the present time an antiserum has been developed against the second virus (tentatively assumed to be the cassava mosaic virus) but not against the brown streak one. The symptoms on the Kenya coast appeared to be more severe than those inland and it is possible that a range of viral strains is involved in the disease. The situation is further complicated in some areas where the green mite *Mononychellus tanajoa* has been recently introduced, in that during dry periods it may be difficult to differentiate between the symptoms of severe mite infection and those of mosaic.

The next two papers by *Peterson and Yang* from McGill University and *Luisoni and co-workers* from the Applied Phytovirology Laboratory in Turin described the work at these institutions involved in trying to identify the causative agent of cassava mosaic. Neither of these workers had made as much progress as *Bock and Guthrie* although the work of both of them indicated that the causative agent was probably a virus or viruses and that affected cassava from West Africa appeared to differ from that originating in East Africa. Both

workers suggested that more study was needed of the vectors particularly in view of the recent demonstration by *Galvez* that the white fly transmitted a doublet virus in *Phaseolus* beans.

Gamborg from the National Research Council in Saskatoon then described his work with *Kartha* on the production of symptom-free material by tissue culture techniques and the possible application of these techniques to further research in this field. Scheduled papers by *Terry* of IITA (concerning a proposal for studying CMD variants) and by *Jennings* of the Scottish Horticultural Research Institute (dealing with breeding for resistance to cassava mosaic) are published in this report but were not presented at the meeting since flight cancellations prevented these participants from attending the workshop.

During the afternoon of February 19 and the morning of February 20 a general discussion was held on the papers presented and following this the participants departed for the Kenya coast to visit experiments laid out by *Seif, Chogoo, Bock, and Guthrie*. The field trip also included a visit to the Coast Research Station at Mtwapa to view the mosaic-tolerant germ plasm collection and to initiate the harvesting of a field trial to assess crop losses due to mosaic in tolerant and non-tolerant lines of cassava.

A summary of the discussions arising from the papers and during the course of the field visit is presented as the final part of this report.

IDRC is grateful to *Dr B. Masiju*, the Director of EAAFRRO for cosponsoring this workshop and for putting the facilities of EAAFRRO at the disposal of the participants. Thanks are also due to *Dr E. Wangati*, the Deputy Director of EAAFRRO, who opened the meeting. *Drs K. Bock* of EAAFRRO/ODM and *T. Chandler* of IDRC's Regional Office in Nairobi acted as joint local secretaries and were responsible for all the local arrangements which ensured the smooth running of the workshop and the field trip.

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Cassava in Kenya

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CASSAVA was introduced into Kenya in the 18th century by the Portuguese, across the Congo Basin from the west and to the shores of the Indian Ocean from the east. It thrived and spread because of its ability to flourish on poor soils and to withstand drought and attacks of pests which make all other crops perish. These assets made it such a valuable famine reserve that the colonial administration prescribed minimum acreages which each farmer was required to plant by law (Jones 1959).

It is used as a major supplementary food to maize and millet which form the main diet of the people. Both tubers and fresh, young leaves are utilized as food, the former either boiled fresh and eaten immediately or dried and ground into flour to make "ugali" (similar to "fufu" in Ghana). The leaves are boiled with fish or pulses to make a sort of curry to the "ugali."

Cassava is not only used as a human food but can also be used as livestock feed. In Kenya the use of chips and broken fresh roots are both practical, although the latter is mostly preferred. In addition the leaves can also be used as fodder during drought when grasses and other feedstuffs become scarce.

Cassava is currently not processed in Kenya, but there is a possibility of a cassava starch factory being constructed at Mariakani, about 40 km from Mombasa.

Production

The production of cassava in Kenya has increased steadily. The quantities produced in individual provinces are given in Table 1. Total production is probably greater than that reported because cassava is grown largely by small-scale

farmers who cultivate small and scattered plots; collection and interpretation of actual production data are therefore difficult.

Today, cassava is grown in nearly every province in Kenya although cultivation is concentrated in the Nyanza, Western, and Coast provinces. The total area under cassava, according to the 1973-74 Ministry of Agriculture provincial annual reports is approximately 58,000 ha, and the total production of dried chips is 408,000 metric tonnes. Average yield varies between 6 and 23 t/ha of fresh tubers depending on cultivars grown and husbandry practiced. Cassava is interplanted with cereals and grown only for local consumption, or as a famine crop, in small plots ranging from less than 1 ha to about 3 ha.

Cassava cultivation is relatively inexpensive and may be attributed to several factors such as low inputs, low labour requirements, and high productivity. Brown (1963) estimated the cost of production of cassava at Ksh. 150/acre, and the return per acre at Ksh. 358-537. At that time approximately 7 Ksh. equalled \$1 U.S.

Agronomy

Cassava is a hardy crop and can thrive and give yields in areas where other food crops would produce little. It grows best where annual rainfall is about 760 mm, though it is often grown in drier areas (Jennings 1970). For maximum production, however, the adoption of proper husbandry practices is necessary. The Crop Production Division of the Ministry of Agriculture, Kenya, has issued the following recommendations to farmers growing cassava:

Table 1. Cassava production in Kenya.

| Provinces and districts of production | Area under cassava (ha) | Metric tons (dry cassava chips) |
|--|----------------------------|------------------------------------|
| Western Province | | |
| Kakamega | 6,727 | 47,089 |
| Bungoma | 2,000 | 14,000 |
| Busia | 12,037 | 84,259 |
| Nyanza Province | | |
| Siaya | 4,283 | 29,981 |
| South Nyanza | 10,958 | 76,706 |
| Kisii | N/A | N/A |
| Coast Province | | |
| Kwale | 2,000 | 14,000 |
| Kilifi | 13,814 | 96,698 |
| Mombasa | 350 | 2,450 |
| Tana River | N/A | N/A |
| Lamu | 75 | 525 |
| Taita/Taveta | N/A | N/A |
| Eastern Province | | |
| Meru | 882 | 5,754 |
| Kitui | 812 | 5,684 |
| Machakos | 280 | 1,960 |
| Embu | 105 | 735 |
| Rift Valley Province | | |
| Elgeyo Marakwet | 417 | 2,919 |
| Baringo | 42 | 294 |
| West Pokot | 13 | 91 |
| Central Province | | |
| Kiambu | 94 | 658 |
| Kirinyaga | 39 | 273 |
| Muranga | 21 | 147 |
| North Eastern Province | N/A | N/A |
| Total | 58,322 | 408,254 |

Land preparation Good deep ploughing and thorough cleaning of the seed-bed is essential. All perennial grasses and weeds should be removed before planting.

Time of planting Normally done during the long rains; however, cassava can be planted during the short rains provided there is sufficient soil moisture to enable the crop to be established.

Planting material Planting material should be selected before harvesting the crop so as to take cuttings only from healthy mosaic-free plants. The recommended varieties for the different provinces are: Coast Province 46106/27 and F279; Nyanza Province 50284/33 and Aipin Valenca; and Western Province 50284/33, 50284/15, 5025/30 and Aipin Valenca. It is recommended that moderately thick cuttings 35 cm long be used and

that these be planted at an angle, with at least 2–3 nodes in the soil. At the coast the crop should be spaced at 1.5 m between rows and 1 m between plants in the row. In other areas, a spacing of 120 × 90 cm could be adopted. Although it is beneficial to plant on ridges, especially at the Coast where the soils are sandy, the work involved in making and maintaining the ridges makes this practice uneconomic.

Fertilization Though application of FYM at the rate of 20 t/ha is recommended for exhausted soils, direct application of fertilizers to cassava is not recommended. However, fertilizer trials conducted at the Coast Agricultural Research Station, Kikambala, have shown that cassava responds significantly to N and in particular to K when applied as 150 kg/ha sulphate of ammonia

and 200 kg/ha each of double superphosphate and muriate of potash.

Harvesting Cassava is normally lifted after 12–18 months in the ground. Before harvesting, the planting material for the next crop should be selected from healthy mosaic-free plants. The above-ground portion of the selected plants should be cut down first and retained for the new planting. The roots should then be carefully lifted with a fork jembe.

Chipping Unless the cassava is to be used immediately after lifting, tubers should be stored in the form of chips. In the preparation of chips, the cassava should be peeled and cut into chips 8 cm long and not less than 3 cm across. Then they should be sun-dried in a single layer on a tray or table. Putting chips in heaps should be avoided since this method encourages mould growth.

Marketing Marketing is done through the Maize and Produce Board and the current price is Ksh. 16/50-kg bag of dry chips (approximately U.S. \$1.90 or \$38/t). There is also a limited market in towns for fresh tubers.

Diseases

Cassava is attacked by various diseases in Kenya, the most important being mosaic. Its wide distribution in the country is primarily due to the free movement of planting material taken from diseased stock from one area to another and also due to the presence of susceptible cultivars in all provinces.

Estimates of yield losses due to mosaic infection vary greatly, examples being 20% in Zaïre, 33–43% in Nigeria, and 83% in Madagascar. In East Africa estimates of yield loss are higher but are complicated by the presence of brown streak disease which is confined to the East Coast of Africa at altitudes below 3500 ft. Complete failure of establishment is common when both diseases are present (Jennings 1970).

Recommended methods of control of mosaic in Kenya are to use cuttings from healthy plants and to rogue infected plants on sight.

Potential as a food crop

Cassava, like many other arable crops in Kenya, is grown as an integral part of the food production system generating the nation's nutritional needs.

As a source of food and energy supply, cassava plays an important role and has a much greater potential than other food crops. Cassava productivity in terms of calories per unit land per unit time is significantly higher than that of other staple food crops: Coursey and Haynes (1970) calculated the production of kilocalories per hectare per day of

some major crops and found that cassava produces 250 kilocalories, compared to rice (176), maize (200), sorghum (114), and wheat (110). Other than high calorie productivity, cassava has the following attributes which make it well suited to small-scale subsistence agriculture: (1) It is easily propagated; (2) It is relatively high-yielding; (3) It is relatively inexpensive to produce as it is easily planted and harvested and requires little or no weeding because of its leafy canopy; it does not have a critical planting or harvesting time; and (4) It is a good risk-aversion crop. Its HCN content makes it subject to minimal animal and pest attacks and it is capable of growing on soils often considered too poor for other crops. Moreover, as a root crop, cassava is biologically more efficient than grain since it does not require an elaborate structure to support its edible portion (63–85% of dry weight of cassava is edible compared with 36% for wheat; Coursey and Haynes 1970; Phillips 1974).

Potential as a cash crop

With an average yield of about 23 t/ha of fresh tubers, cassava has the potential of being one of the important cash crops in Kenya. According to the statistics provided by Phillips (1974), in 1962 demand for cassava by the EEC countries was 413,704 tonnes; by 1971 the market had expanded to 1.5 million tonnes, an increase of 363%. In 1973 demand for cassava is estimated to have been approximately 1.9 million tonnes. With such a growing demand for cassava by feed compounders in EEC countries, Kenya cassava has a definite export potential. Factors which favour Kenya as an exporter of cassava are as follows: (1) A high hectareage under cassava which is underutilized. As it is only grown as a famine crop, there is plenty of room for expansion; (2) The low cost of production, due to relatively cheap labour costs; and (3) Kenya is closer to the European markets than are the Asian exporting countries and freight charges are favourable.

The potential of cassava as a food and cash crop in this country is considerable although disease prevalence, particularly mosaic, and the marketing structure seem to be major constraints limiting large-scale production. Were these two problems resolved, cassava could have a bright future in Kenya.

Acknowledgments

We wish to thank Dr K. R. Bock and Dr E. J. Guthrie of EAAFRo for their advice and encouragement throughout this exercise. This paper is published with the permission of the

Director of Research, Ministry of Agriculture, Kenya.

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Recent Advances in Research on Cassava Viruses in East Africa

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Introduction

WE are studying three different aspects of cassava viruses at EAAFRO and in the field at the Coast Agricultural Research Station, Mtwapa, where we have collaborated with A. A. Seif, Plant Pathologist, Kenya Department of Agriculture, and his colleagues. The aspects are: (1) The establishment, under glass, of a mosaic-free collection of cassava varieties of direct or indirect importance to East African agriculture; (2) Studies on the epidemiology of cassava mosaic in the field; and (3) The identification and characterization of cassava mosaic and cassava brown streak viruses.

The EAAFRO mosaic-free collection

At the conclusion of a 25-year cassava breeding program undertaken by EAAFRO, H. H. Storey selected approximately 90 lines which either were promising commercially or would be of outstanding importance in further breeding. When we surveyed cassava collections in East Africa in 1969–70, it was clear that much of this material was in danger of being lost, or of becoming totally infected with mosaic.

We therefore collected apparently healthy material of as many of the EAAFRO varieties as possible, whenever this was opportune, during visits to various field stations in East Africa. We also included material of the popular varieties of the different areas visited.

The reason for initiating the collection was both to safeguard the existence of what is obviously valuable breeding material, and also to enable

EAAFRO to supply mosaic-free cuttings on request to agronomists and breeders.

While most of the work involved routine horticultural techniques, of interest is our observation that mosaic infection can on occasion remain latent through two propagation cycles. Material collected in the field as apparently free of mosaic remained so for two successive cycles of propagating by cuttings, only manifesting itself in the third cycle. One possible explanation is that we collected material of very recent infection, and that infection remained sub-clinical because of the comparatively rapid succession (2–3-month intervals) of propagating cycles.

Studies on the epidemiology of cassava mosaic

In our field studies we set out first to compare the rate of spread of mosaic within plots which spread into plots planted initially with mosaic-free material. We would stress that all this work has been of an observational, as distinct from a statistical, nature.

For studying the rate of spread within plots, seven centrally placed, mosaic-infected cuttings of cv. 46106/27 were surrounded by concentric hexagons of a total of 156 mosaic-free cuttings of the same variety, which is described as moderately tolerant, but not resistant, to mosaic (Plot 1). Plants which became infected during the experiment were not rogued. Spread from infected to healthy plants was rapid and at the time of harvest 14 months after planting, 84 of the 156 plants (54%) were infected.

In contrast, the amount of spread into an isolated (300 m from nearest cassava) mosaic-free plot (Plot 2) was very small. Here, 100 mosaic-free plants each of cv. 46106/27 and the highly susceptible F279 were planted in 10 alternate rows of 20 plants; plants were 1 m apart with 2 m between the double rows. Infected plants were rogued. At the time of harvest (14 months) only two of the 100 plants of 46106/27, and 15 of the F279 (15%) plants, had become infected. Of the latter, there is evidence that at least eight were infected before transplanting to the field; 7% infection of F279 for the 14-month period is possibly a more accurate figure.

These results suggest that satisfactory field control of mosaic might be achieved by the use of mosaic-free propagation material resistant cultivars planted in reasonable isolation, with rigorous roguing of infected plants.

In order to ensure that these results were not attributable to site ecology, four further plots, each similar to the second experiment (Plot 2) described above, were initiated in 1975 in areas of widely differing ecologies. These were:

Plot 3: on same site as plot 1, in open grass fields and with some degree of isolation from other infected cassava. Shortly after initiation of this trial several plots of cassava, in which incidence of mosaic was moderately high, were planted within 50 m of plot 3;

Plot 4: surrounded by cashew trees;

Plot 5: sheltered from the prevailing southeast monsoon wind by citrus, cashew, and coconut;

Plot 6: on a farm near Mtwapa Research Station, surrounded by widely spaced coconut palms.

Spread of mosaic into these plots was very slow and the disease did not build up at any time during the season. Results are summarized in Table 1.

TABLE 1. Incidence of mosaic in "mosaic-free" plots.

| | 46106/27 | F279 | |
|----------|----------|-------|--------|
| Plot 3 | 0/100 | 0/100 | |
| Plot 4 | 2/100 | 5/100 | |
| Plot 5 | 4/100 | 2/100 | |
| Plot 6 | 0/100 | 2/100 | |
| Total: | 6/400 | 9/400 | 15/800 |
| Percent: | 1.5 | 2.3 | 1.9 |

Crop-loss assessment trial To estimate the effect of planting infected cuttings on yield of 46106/27 and F279, a line of 35 plants derived from infected cuttings was planted between two lines of 35 plants derived from mosaic-free cuttings. Rows were 2 m apart with 1.5 m between

plants. The trial was established in May 1975 and lifted in February 1976 (at 10 months).

Analysis of variance showed the results to be highly significant in level of loss between the varieties: the drop in yield due to disease was greater for F279 than for 46106/27 (Table 2).

TABLE 2. Mean yield (untransformed data) per plant (in kilograms) of plants derived from infected and healthy cuttings.

| | 46106/27 | F279 |
|--------------|----------|------|
| Healthy | 3.86 | 3.67 |
| Diseased | 1.19 | 0.52 |
| Percent loss | 70 | 86 |

Identification of CMD and brown streak viruses

We have isolated by sap transmission from cassava to *Nicotiana clevelandii* two morphologically similar and serologically related but distinct viruses. In spite of repeated attempts, we have been unable to infect cassava with either; until this has been done their identity must remain open to question.

We have tentatively concluded that one of the two viruses is likely to be cassava brown streak (CBSV) for reasons which we now give, and we refer to it throughout the text as CBSV. The second virus is either a strain of CBSV, and the symptoms of brown streak are masked by cassava mosaic virus or infection in the cultivars studied is symptomless, or it is in fact cassava mosaic virus (CMV). We have some evidence to support the latter hypothesis, and refer to the virus in the text as CMV.

Particle morphology Purified preparations of both viruses contain numerous similar isometric particles, 20 nm in diameter, which are usually paired (30 × 20 nm). They are indistinguishable from particles of maize streak virus, but are apparently unrelated to MSV serologically.

Host range and symptomatology (Fig. 1-3) In 10 days CBSV induces in *Nicotiana clevelandii* a systemic vein clearing followed by an extremely severe systemic leaf crinkling and curling, with reduction in size of the leaves. Subsequently the leaves develop a fine necrotic vein etch. CBSV also infects *Petunia hybrida*, *Datura stramonium*, *N. glutinosa*, *N. rustica*, and *N. tabacum* (hosts also infected by Lister (1959) in his work on CBSV), *D. ferox*, *Solanum nigrum*, and *Salpiglossis sinuata*.

CMV induces systemic leaf curling and crinkling in *N. clevelandii* in 12-14 days, which is markedly less severe than that induced by CBSV. Leaf size is only moderately affected; coarse irregular yellow vein banding and yellow areas

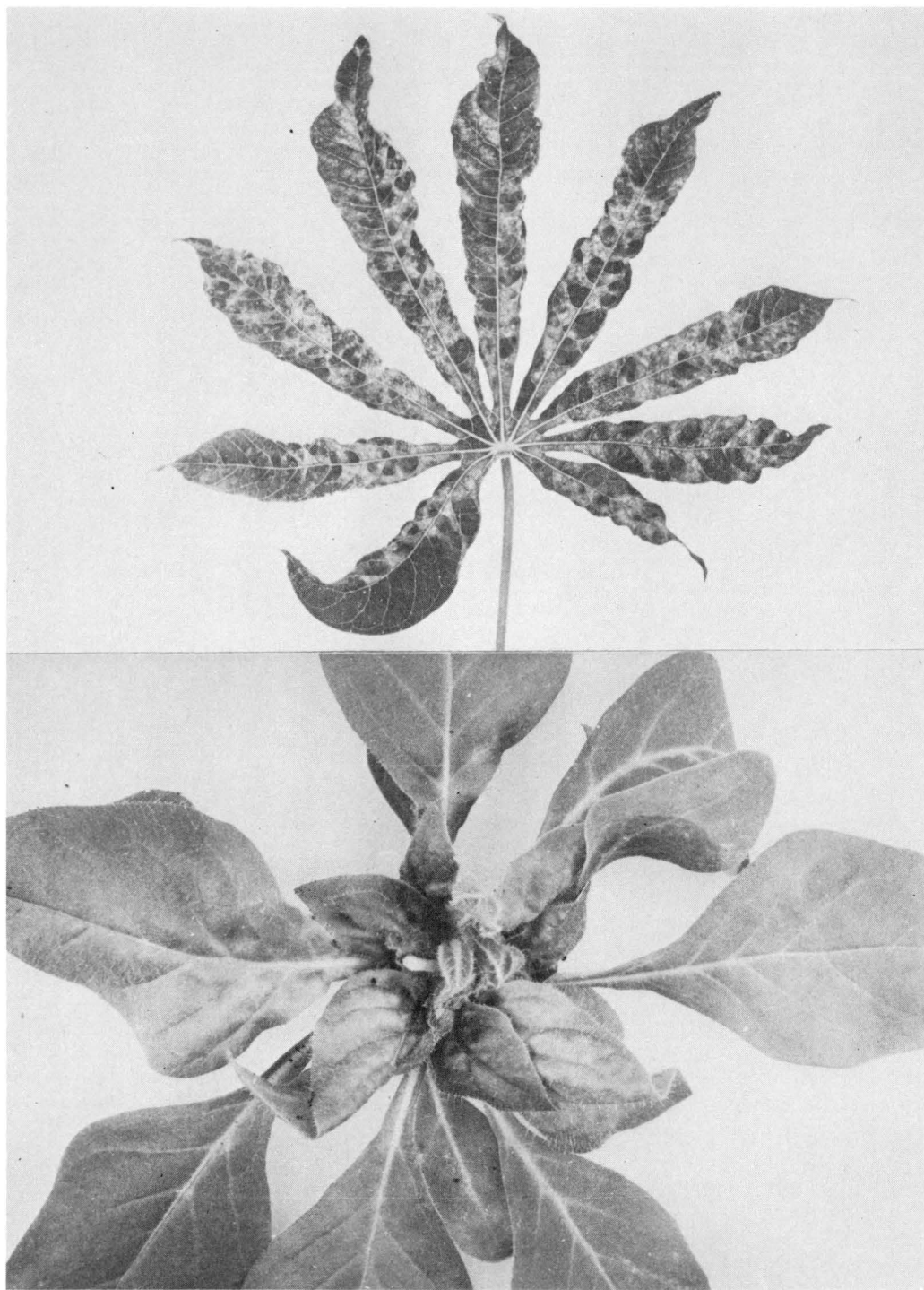


Fig. 1. Top Symptoms of African cassava mosaic in cassava; *Bottom* *Nicotiana clevelandii* systemically infected with a virus isolated from African cassava mosaic material.

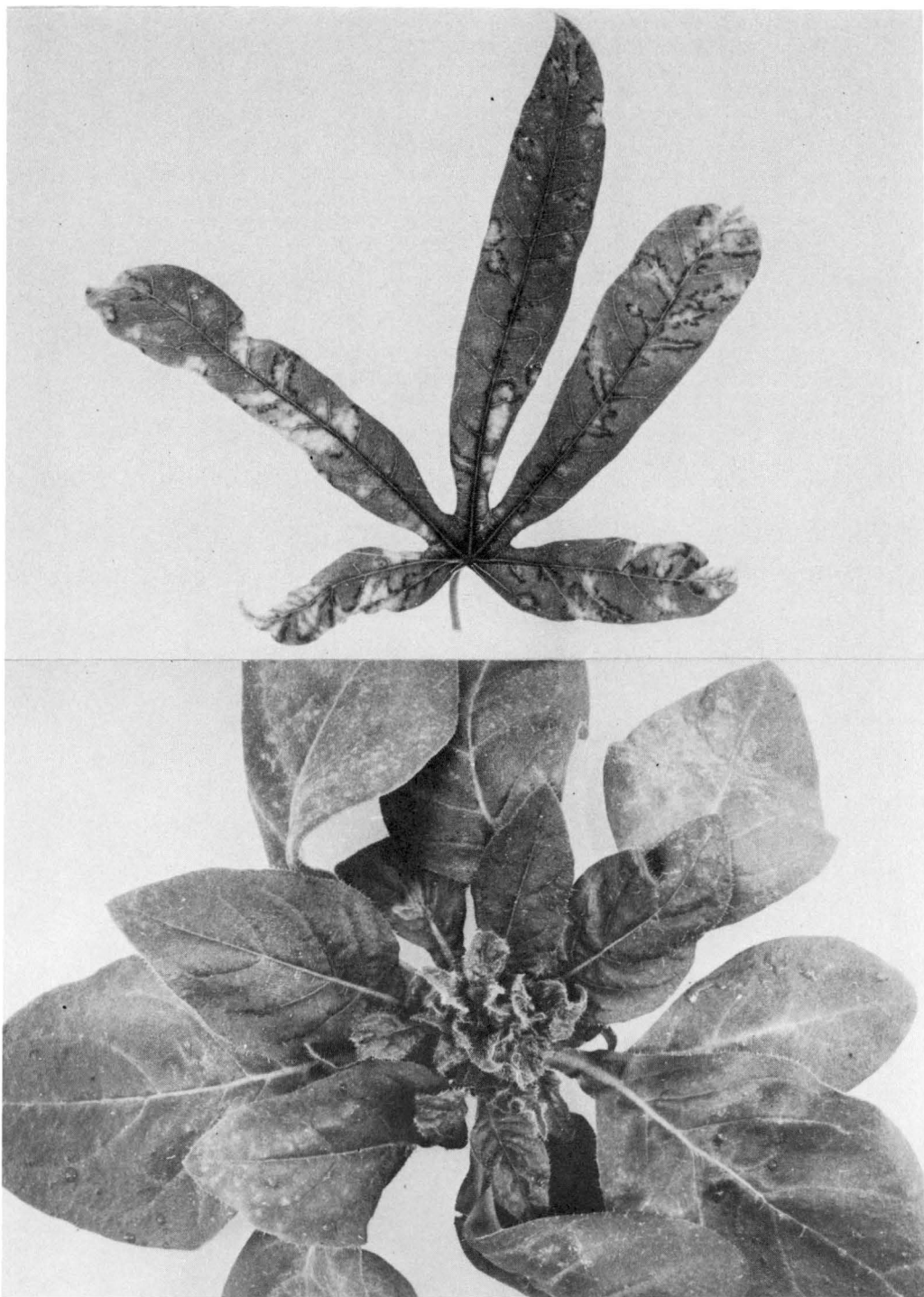


Fig. 2. *Top* Symptoms of cassava brown streak virus in cassava; *Bottom* *Nicotiana clelandii* systemically infected with a virus isolated from cassava brown streak virus material.



Fig. 3. Top Particles of the virus (approx. 19×33 nm) isolated from African cassava mosaic. The particles of brown streak are morphologically identical (Micrograph by Scottish Horticultural Research Institute, Invergowrie, Dundee, Scotland); *Bottom* Reaction of the highly susceptible variety F279 to cassava mosaic. Infected line at centre, healthy F279 to right and left.

develop and are typical of infection. Host range is essentially similar to that of CBSV, but we were unable to infect *S. nigrum* or *S. sinuata*.

Purification CMV is easily purified from systemically infected *N. clelandii* by homogenizing leaves in 0.1 M borate buffer containing 1% mercapto-ethanol at pH 8.1, and clarifying with equal volumes of a 1:1 mixture of *n*-butanol and chloroform. For biochemical assays for RNA and protein entity determination, the pellet derived from the first ultracentrifugation of clarified extracts was resuspended in 0.005 M borate containing 0.005 M ethylenediaminetetraacetic acid and 0.2% formalin; pellets derived from the two subsequent ultracentrifugation cycles were resuspended in 0.005 M borate containing 0.005 M EDTA. Polyacrylamide gel electrophoresis studies by Dr H. Barker (Scottish Horticultural Research Institute) indicated that CMV, like maize streak virus, contains one species of protein and two of RNA (mol wt 34000, 1.7×10^6 and 1.3×10^6 , respectively).

This method of purification did not prove satisfactory for antiserum preparation; an antiserum with a comparatively low titre (1/64 in agar gel diffusion tests) was prepared, however, and was used to test the relationship of CMV to CBSV. Purification methods suitable for CMV are unsuitable for CBSV, thus underlining the host reaction and serological differences between the two.

Serology We tested purified CBSV and CMV viruses against CMV antiserum. CMV reacted to a

titre of 1/64, and there was spur formation between CBSV and CMV, suggesting that the viruses are closely related. The antiserum did not react with crude clarified sap of either CBSV or CMV.

Geographic origin of isolates and association with cassava virus symptoms We have isolated CMV from mosaic-infected material (seven isolates) collected from widely separated areas throughout East Africa (in Kenya, Uganda, and Tanzania), but we have not isolated the virus from CBSV-infected or apparently healthy material.

CBSV has been isolated from cassava showing classic CBSV symptoms but without mosaic (four isolates) and from plants with a dual infection of CBSV and CMV, all but one originating from coastal areas of Kenya and Tanzania. The exception (variety No. 20, from Ukiriguru, W. Tanzania) was collected by Dr D. L. Jennings as CBSV-infected material.

Summary

We have isolated two serologically related but distinct viruses from cassava infected with brown streak and with mosaic. Our working hypothesis, which is admittedly based at the moment on tenuous data, and which can only be verified by re-inoculation to cassava, is that one is cassava brown streak virus and the other mosaic virus.

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Characterization Studies of Cassava Mosaic Agents

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Introduction

It has been estimated (Nestel and MacIntyre 1973) that up to 300 million people in the world's tropical areas are heavily dependent on cassava as a major carbohydrate source. The relative ease of cassava cultivation has probably been an important factor in the development of a variety of uses of cassava. In addition to use in several food products, the starch is used for some industrial applications, and recently it has entered world trade as an export from Thailand to EEC countries where it is used in livestock feeds (Phillips 1974).

Among the known problems and pests affecting cassava, viruses have long been considered an economic threat. The best-known viruses are those occurring in Latin America, where three or four cassava virus diseases, including cassava common mosaic virus, a potato-virus \times -like particle, have been identified and partially characterized by Costa and Kitajima (1972).

The Latin American viruses are not regarded as serious economic problems provided that they are controlled by proper management, use of clean material for propagation, and that they are not supplemented by other viruses. Thus a strict quarantine is maintained against cassava importation.

In Africa, a virus disease known simply as cassava mosaic (CMD) causes estimated worldwide losses of 30–50%; in West Africa, where most of the generally used cultivars are infected, losses may range from 30 to 80% or more (Hahn and Howland 1972). African cassava mosaic differs from the Brazilian cassava common mosaic in that the former is: (1) present in many widely used cultivars, and readily disseminated by

vegetative propagation; (2) not readily mechanically or manually transmissible; and (3) transmitted in the field by the "sweet potato whitefly," *Bemisia tabaci*, and regarded since the work of Storey and Nichols (1938) as a classic example of a whitefly transmitted virus.

Chant (1958), who studied the vector-virus relationship, noted that whiteflies required at least 4 h of feeding on young cassava leaves, followed by a 4-h incubation period, before they could transmit the virus to subsequent plants, and that adult whiteflies remained infective for 48 h or more. Chant also tried to infect a number of related (Euphorbiaceae) genera with cassava mosaic virus, both by whiteflies and by mechanical inoculation, with negative results. The general consideration that the causal agent of cassava mosaic is not readily transmissible by mechanical inoculation has effectively discouraged work on it.

Recently, however, Bock et al. (1974) and Bock (1975) have managed to transmit two infectious agents manually from cassava to *Nicotiana clevelandii*, from which they have extracted and purified preparations containing small (20 m μ) isometric particles, usually occurring in pairs. So far, attempts to infect cassava with these particles have proven negative, so that their identification as cassava mosaic and brown streak viruses is tentative. However, similar paired particles have recently been shown in association with maize streak disease, sugar beet curly top disease (Mumford 1974), and bean golden mosaic virus (Galvez and Castaño 1975). The "doublet" particles of bean golden mosaic virus are infective only when they remain paired.

The remainder of this paper deals with our

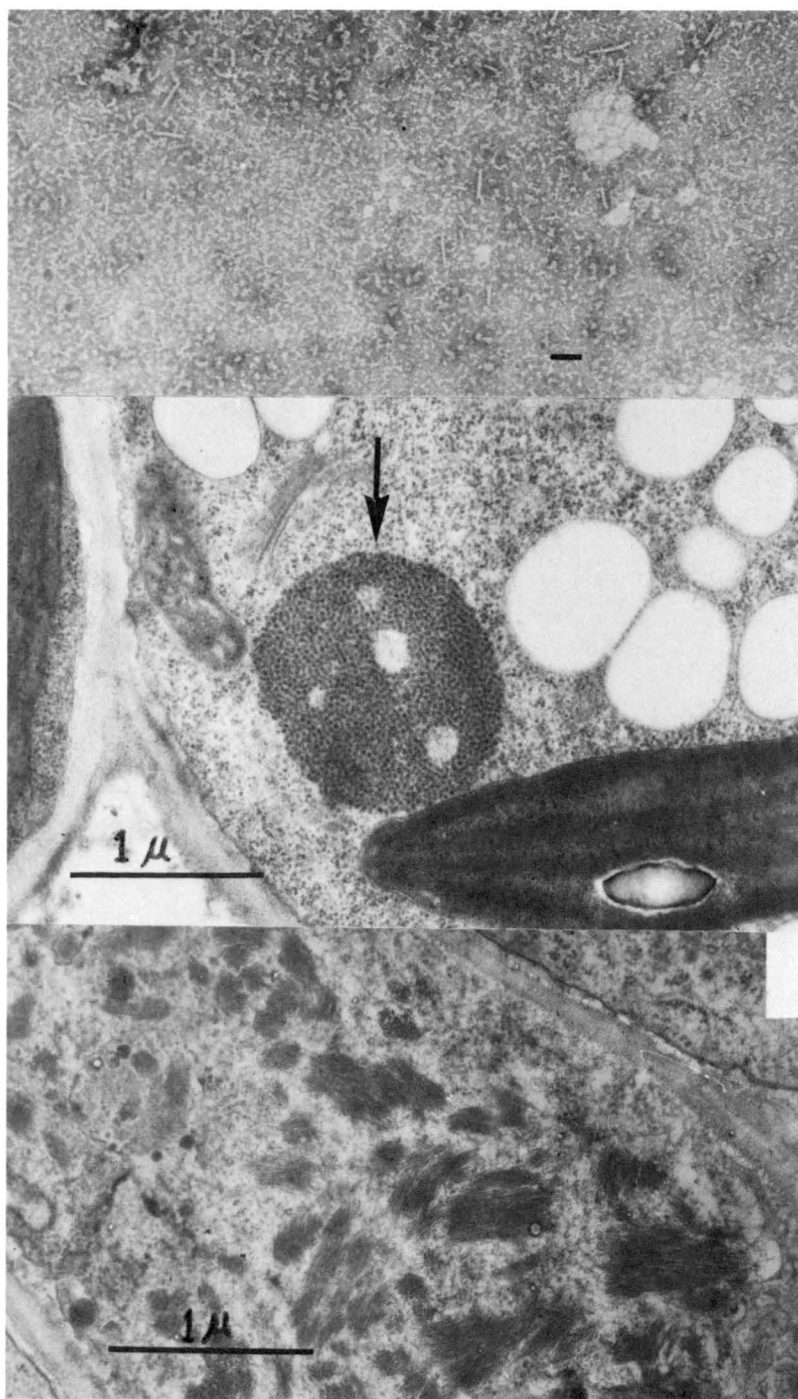


Fig. 1. (top) Rod-shaped fragments and miscellaneous debris from a "purified" extract (calibration bar = 100 m μ). (middle) Sectioned leaf material; densely stained aggregate of small spherical particles (arrow); measurements made on a higher magnification print suggest a diameter of approximately 20 m μ . (bottom) Sectioned young leaflet; aggregates of flexuous rod-shaped particles.

attempts at identification of possible agent or agents involved in the infection of our Nigerian cassava clones.

Transmission

During the early part of our work, no healthy uninfected "control" material was available, so conventional manual inoculations were made from cassava to various herbaceous hosts, with the objective of finding an alternate host which might serve as either a bioassay host or a more convenient source of tissue for extraction and purification studies. The following plants were tried as hosts: tobacco (various varieties), cucumber (reported as a host to which whiteflies can transmit—(Menon and Raychaudhuri 1970), *Gomphrena globosa*, *Datura stramonium*, *Chenopodium amaranticolor* (*C. quinoa*), *Lychnis divaricata*, *Petunia hybrida* (a potential check for presence of brown streak agent—(Lister 1959), Hollyhock and Abutilon (both reported hosts of other whitefly-transmitted viruses), Sweet pepper, Zinnia, Tomato, *Physalis floridana*). None of these plants ever showed any sign of symptom development.

Dodder transmission, generally unsuccessful for whitefly transmitted viruses (Costa 1969), has also been attempted. One castor bean plant, reported as a host which whiteflies can infect (Singh IITA, personal communication) did develop symptoms which were subsequently graft transmissible to additional castor bean plants.

Purification

Various purification procedures were undertaken, using infected cassava leaves as source material, after obtaining some healthy (i.e. symptom-free) cassava lines of CIAT origin; healthy and infected CIAT varieties were obtained by graft transmission from our African material.

In consideration of the possible involvement of a viroid in the African disease, an opinion of some workers (Galvez 1974, personal communication), nucleic acid extraction and subsequent polyacrylamide gel electrophoresis were used, in procedures similar to those applicable for the potato spindle tuber viroid (T. J. Morris, personal communication). No consistently obtainable "viroid-like" RNA component was detected. However, some nucleic acid extracts have shown signs of infectivity on *Gomphrena globosa* and *Nicotiana clevelandii*, the latter of which has also shown itself very susceptible to contamination with unwanted viruses.

Many extraction/purification schemes, using various buffer systems and clarification methods, have been used in our attempts to detect virus or

virus-like particles in extracts from cassava. As the usual manner of evaluation of purification methods, i.e., assaying the preparations after each step for infectivity by manual inoculation of test plants, has not been applicable, we have evaluated our methods on the basis of the presence or absence of any components specific to infected material after density gradient centrifugation (or electrophoresis) of the processed extract. Samples from centrifuged gradient columns have been examined by electron microscopy when appropriate, if scheduling was possible. Material concentrated and pelleted by high speed centrifugation has sometimes been assayed for manually transmissible or injectable infectivity on test plants, or assayed similarly after nucleic acid extraction.

A detailed description of all the methods tried is given in Appendix A. Some of the approaches used appear to warrant a brief mention. Various detergents and solvents were evaluated for their ability to dissolve or disperse the milky "latex" substance(s) present in cassava leaves and petioles. Similarly, various additives/adsorbents have been used for clarification of the initial extract.

Some extractions have involved the use of electrophoresis as a possible method of separating any components which might appear to be homogeneous with respect to sedimentation behaviour in sucrose density gradients. Thus occasionally material was layered onto a sucrose density gradient column which was then subjected to electrophoresis in a laboratory-built apparatus, rather than centrifuged in the usual manner. Extracts have also been subjected to electrophoresis on 3% polyacrylamide gels, an approach which has been useful for some "spherical" viruses. Because roots are often overlooked when such striking visual leaf symptoms are present, a few extractions have been made using root tissue as source material.

We have found that Triton X-100, an alkylaryl polyether alcohol which has been found useful for decreasing aggregation of some viruses during purification procedures, dissolved or dispersed the "latex" in cassava extracts, while the detergents Leonil SA, Igepon, and SDS were less effective. *n*-butanol, commonly used during clarification of initial extracts, also dissolved or broke down the latex to some extent. Extraction of leaf or petiole tissues in low salt-low pH buffers, followed by *n*-butanol clarification, has usually provided reasonably well-clarified extracts for high speed centrifugation. At higher pHs, as expected, less green material is removed from the initial extract, but the addition of Triton X-100 to 1% keeps the pigmented material dispersed well enough so that a

useable high-speed pellet can still be obtained.

Unfortunately, none of these attempts, as yet, have enabled us to consistently detect any component specific to infected cassava. Furthermore, our purification results have often been rather variable, and it has been difficult to repeat some of them. The most common ingredients in many of our preparations have been short fragments of rod-shaped particles having a rather coarse helical substructure, which are present in extracts from both healthy and infected material (Fig. 1 *top*). Small, somewhat variable "spherical" particles, which may represent fraction 1 protein, have also been encountered on electron microscope examination of our "purified" preparations.

It is possible that our preparations at times may have contained particles which would sediment with, and be masked by, normal host ribosomes, as the sedimentation coefficients of some of the recently reported paired pentagonal virus particles are close to those of ribosomes. It is also possible that we are losing any virus present during our purification procedures through adsorption to host contaminants or precipitation by them; the extracts from cassava certainly seem to contain an abundance of interfering materials.

Electronmicroscopy

Leaf dips of infected or healthy leaves, after negative staining with sodium phosphotungstate, have shown the presence of long, slightly flexuous rod-shaped particles having the same coarse helical substructure as the shorter fragments shown in Fig. 1 (*top*).

Examination of thin sections of older leaves, after fixation and embedding by standard methods, has occasionally revealed the presence of densely stained aggregates of small spherical particles, having a diameter approximating that of ribosomes (Fig. 1, *middle*). Bundles of long rod-shaped, slightly flexuous particles have also been encountered.

Samples taken from younger leaves showed a few cells containing an abundance of flexuous rod-shaped particles, in various arrangements (Fig. 1, *bottom*), having a transparent central channel, a diameter of about 10 μ , and a hexagonal arrangement, when seen in cross-section at higher magnification. Cells containing an abundance of vesicles were also observed.

Although the general appearance of these particles resembles that of various known flexuous rod-shaped viruses, it also bears a striking resemblance to rod-shaped particles occurring as "slime" or P-protein, often found in sieve

elements (Steer and Newcomb 1969). Our observations, with respect to rod-shaped particles, also fit the descriptions given by Murant et al. (1974), who have also examined embedded cassava leaves, and who have observed rod-shaped particles as apparently normal constituents of healthy cassava.

We have encountered neither mycoplasma-like nor rickettsia-like organisms in our sectioned material.

Summary

We have not as yet been able to consistently implicate an agent responsible for the symptoms shown by our cassava. Since we have obtained signs of infectivity on *N. clevelandii*, we hope to try to expand our collection of it and try the approaches used successfully by Drs Bock and Guthrie. We intend to continue our electronmicroscope investigations, as some of the particles we have occasionally encountered (e.g. Fig. 1, *middle*) which may or may not be virus, have apparently not been observed in cassava by other investigators. Although the symptoms are typical of those associated with cassava mosaic disease, it is possible that our cassava is afflicted with a mixture of agents, some of which are latent.

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Appendix I — Abbreviation Key

H. S. = high speed
 L.S. = low speed
 d. g'd't = d. grdt. = density gradient
 2-ME = 2-mercaptoethanol
 clarif. = clarification
 grdt = g'd't = gradient
n-buOH = *n*-butanol
 pel't = pellet
 N.A. = nucleic acid
 lvs = leaves
 DIECA = Diethyldithiocarbamate

Purification Trials

| | | |
|----------------|---|---|
| 13 Jan. | 40 g frozen lvs. 0.2 M NaAc ⁻ -HAc ⁻ , 0.1 M EDTA, pH 5.4 H.S. pel't d. g'd't. | Light scattering bands, but no UV absorbance at 254 mμ. |
| 14-15 Jan. | Repeat of above. | Bands in D extract, not present in H. In E.M., mixture of rods and cellular debris. |
| 16 Jan. (a) | 80 g lvs. 0.01 M NaAc ⁻ -HAc ⁻ buffer, low salt pH 4, 1% 2-ME, <i>n</i> -buOH clarif., H.S. low pH pel't, d. gdt. | Possible light scattering zone specific to D. |
| (b) | 30 g frozen lvs. 0.5 M borate, pH 8.2, & 1% 2-ME, <i>n</i> -buOH clarif., followed by addition of 1% Triton. H.S. pel'ts, d. grdt. | D & H extracts about the same, by both light scatter and 254 mμ O.D. fractiona- tion. |
| 19-20 Jan. | 80 g lvs. Phosphate-citric acid buffer, low salt pH 4.15 and 1% 2-ME, <i>n</i> -buOH clarif., low pH H.S. d. grdt. | D & H gdt. same by light scatter |
| 21 Jan. | 45 g lvs. Repeat 16 Jan. (a). | Broad light scattering zone, slowly sedimenting, present in D extracts but not healthy. |

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|---------|--|---------------------|--|
| 27 Jan. | 70 g lvs. 0.01 NaH_2PO_4 buffer, pH 3.8 & 1% 2-ME, <i>n</i> -buOH clarif., H.S. pel't. d. grdt. | | No zones specific to D. |
| 28 Jan. | 48 g lvs. 0.1 M K_2HPO_4 , pH 8; & 1% 2-ME, <i>n</i> -buOH clarif., H.S. pel't, d. grdt. | | Light scattering zones; small spherical particles, some paired in E.M. |
| 5 Feb. | 100 g lvs. 0.01 M NaAc^- - HAc^- buffer, pH 4, & 1% 2-ME, <i>n</i> -buOH clarif., H.S. pel't, d. grdt. electrophoresis. | | One light scattering band present in both D & H extracts |
| 10 Feb. | 70 g lvs. Repeat above. | | Same as above |
| 13 Feb. | 75 g lvs. 0.2 M NaAc^- - HAc^- buffer, pH 4.5, & 1% 2-ME; clarify by overnight incubation in cold room. H.S. pel't, d. grdt. | | D & H extracts contained same single, slowly sedimenting zone. |
| 17 Feb. | 50 g lvs. 0.1 M citric acid-sodium citrate buffer, pH 6, & 1% 2-ME, <i>n</i> -buOH clarif., H.S. pel'ts, d. grdt. | | No zones in D or H extract |
| 19 Feb. | 65 g lvs. 0.1 M borate, pH 8.2 & 1% 2-ME, <i>n</i> -buOH clarif., followed by addition of Triton to 1%. H.S. pel't, <i>n</i> -buOH clarif. of pelleted material. D. grdt. centrifugation & electrophoresis. | | Nothing distinctively specific to D extract observed. |
| 25 Feb. | 15 g petioles. 0.01 M NaAc^- - HAc^- buffer, pH 4, & 1% 2-ME. L.S. clarif. H.S. pel't, d. grdt. | | Light scattering zones, not "specific" to D, & absorbing poorly at 254 $\text{m}\mu$. |
| 26 Feb. | 70 g lvs. 50 g petioles. 0.01 M NaAc^- - HAc^- buffer, pH 4, & 1% 2-ME, <i>n</i> -buOH clarif., H.S. pel't, d. grdt. | | D petiole extract contained a zone which looked like some sort of precipitate; max. UV absorbance at 275 $\text{m}\mu$. |
| 4 Mar. | 60 g lvs. 30 g petioles. 0.01 M citric acid-sodium citrate buffer, pH 8, 0.01 M Na_2SO_3 & 0.1% 2-ME, <i>n</i> -buOH clarif., followed by addition of Triton X-100 to 1%. H.S. pel't, d. grdt. | | Band of slowly sedimenting material at top of each gradient, nothing specific to D extracts. |
| 11 Mar. | 60 g lvs. 40 g petioles. 0.01 M citric acid-sodium citrate buffer, pH 8. 0.01 M Na_2SO_3 , 0.1% 2-ME, <i>n</i> -buOH clarif., 1% Triton X-100; H.S. pel't, d. grdt. | high pH low salt | Nothing specific to D extracts; material remaining near top of gradients in all extracts. |
| 12 Mar. | 40 g lvs. 40 g petioles. 0.01 M glycine-HCl buffer, pH 3.8, 0.01 M Na_2SO_3 , 0.1% 2-ME, <i>n</i> -buOH clarif., followed by addition of Triton X-100 to 0.5% H.S. pel't, d. grdt. | low salt low pH | Some material remained at top of gradient; no zones. |

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| 17 Mar. | 47 g lvs. 40 g petioles; same as above. | | One band, near top, of each gradient; none specific to D. |
| 18 Mar. | 60 g lvs. 25 g petioles. 0.1 M glycine-NaOH buffer, pH 8 and 0.5% 2-ME, <i>n</i> -buOH clarif., H.S. pel't, d. grdt. | high pH | Single band, near top, in all extracts. |
| 19 Mar. | 40 g frozen lvs. 0.1 M Na ₂ HPO ₄ , 0.1% Na-Thioglycollate, 0.01 M DIECA. L.S. clarif, followed by precipitation with 5% polyethylene glycol. Resulting precipitated material subjected to phenol-SDS extraction for nucleic acids. Final nucleic acid prep. subjected to polyacrylamide gel electrophoresis. | | Nothing specific to D extract. |
| 24 Mar. | 35 g lvs. 35 g petioles. 0.05 M K-PO ₄ buffer, pH 7, & 1% 2-ME, with 25% sucrose; <i>n</i> -buOH clarif., H.S. pel't, d. grdt. | | Diseased and healthy petiole extracts and D & H leaf extracts resembled each other. |
| 8 Apr. | 30 g lvs. 40 g petioles. 0.02 M borate buffer, pH 8.5, 0.01 M Na ₂ SO ₃ & 1% 2-ME, <i>n</i> -buOH clarif., followed by addition of Triton X-100 to 1% extract divided for grdt. centrifugation & infectivity assay. | low salt high pH | Nothing specific in D extract by either light scatter or fractionation and UV scanning at 254 mμ. |
| 9 Apr. | 30 g lvs. 34 g petioles. 0.02 M borate buffer, pH 8.5, 0.01 M Na ₂ SO ₃ , 1% 2-ME. Clarif. by short H.S. centrif., followed by addition of Triton X-100 to 1%. H.S. pel't, d. grdt. centrif. | low salt high pH | Same as above. |
| 14 Apr. | N.A. extraction of butanol chloroform clarified extract; infectivity assay on <i>Gomphrena</i> → 1.1., not transferable. | | |
| 23 Apr. | 32 g lvs. 30 g petioles. 0.01 M citric acid-sodium citrate buffer, pH 8, 0.01 M Na ₂ SO ₃ , 1% 2-ME, <i>n</i> -buOH clarif., H.S. pel't, d. grdt. centrif. & gel electrophoresis. | | No zones. |
| 29 Apr. | 100 g root tissue. 0.01 M citric acid-sodium citrate buffer, pH 8, Na ₂ SO ₃ , 1% 2-ME. L.S. clarif., H.S. pel't. Sucrose grdt & gel electrophoresis. | | No bands |
| 15 May | 15 g lvs. 0.02 M KPO ₄ buffer, pH 8, 0.01 M DIECA, 0.01 M nathioglycollate, L.S. clarif., followed by addition of hydrated calcium phosphate (HCP) & L.S. centrif. for clarif., H.S. pel't, d. grdt. | | HCP → good clarification of crude extract, but no zones obtained in d. gdt. |

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| 16 May | 56 g lvs. 0.01 M citric acid-sodium citrate buffer, pH 8, 0.01 M Na ₂ SO ₃ , 1% 2-ME, <i>n</i> -buOH clarif., followed by addition of Triton X-100 to 1%. H.S. pel't, d. grdt. | high pH low salt | Nothing specific to D, H & D extracts showed very similar fractionation patterns. |
| 16 May | Gel electrophoresis of stored root nucleic acid extracts. | | No bands. |
| 23 May | 20 g young lvs. nucleic acid extraction; polyacrylamide gel electrophoresis. | | Nothing specific to D extract. |
| 30 May 4 June | Same. | | Same. |
| 9, 11-13 June | 40 g lvs. & petioles. Tris-glycine buffer, pH 8.9 & 1% 2-ME. Add Triton X-100 to 1%, H.S. pel't. Run on gel electrophoresis. | | Extra, slowly migrating component present in D extract. |
| 16, 19 June | Virus gel work. | | No differences noted in gels, with respect to faster migrating components. Some minor differences in slower components noted, inconsistent and variable. |
| 21 June 2 July 4, 7 July 10, 15, 16, 21, 23, 31 July | Virus gel work. | | |
| 12 Aug. | 36 g lvs. 0.1 M citric acid-sodium citrate, pH 8, .01 M Na ₂ SO ₃ , 0.1% 2-ME L.S. clarif., H.S. pel't. Divided for infectivity assay (by injection into <i>N. clevelandii</i>) & d. grdt. | | No specific zones or bands in healthy. |
| 14 Aug. | 50 g lvs. 0.2 M NaAc ⁻ -HAc ⁻ , 0.1 M EDTA, pH 5.4, 1% 2-ME. L.S. clarif. H.S. pel't, d. grdt. | | Diffuse, broad "zone," about halfway down column, in both D & H extracts. |
| 15-17 Sept. | 20 g lvs. nucleic acid extraction & infectivity assay, & polyacrylamide gel electrophoresis. | | Two "extra" minor slowly migrating bands in disease extract; thought lacking in healthy extract because of lower concentration. |
| 17 Sept. | 46 g lvs. & petioles. Ground in 0.1 M sodium citrate buffer, pH 8 & 0.01 M Na ₂ SO ₃ , 0.1% 2-ME. L.S. clarif., followed by addition of Triton X-100 to 1%, & H.S. pelleting. Pelleted preparation divided & used for infectivity assay, & nucleic acid extraction, followed by polyacrylamide gel electrophoresis. | | No bands observed. No symptoms developed in injected <i>Nicotiana clevelandii</i> . |

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| 22 Sept. | 45 g lvs. Ground in 0.1 M KPO_4 buffer, pH 7. 0.5% 2-ME, 10% polyvinyl pyrrolidinone (PVP) clarif. by L.S. centrif., followed by <i>n</i> -buOH treatment. Preliminary trial with ammonium sulfate precipitation. | Addition of ammonium sulfate to clarified extract to 30% → precipitate, assayed for infectivity in <i>Gomphrena</i> , petunia. No symptom development. |
| 23–24 Sept. | 60 g leaves & petioles: Repeat of above, but without addition of PVP as adsorbent during clarif. | Addition of ammonium sulfate to 30% → no precipitate. |
| 30 Sept. 1 Oct. | 30 g lvs. & petioles. 0.02 M NaPO_4 buffer, pH 7, 0.01 M DIECA. Clarif. by <i>n</i> -buOH treatment. Preparation divided, half put into H.S. pel't run directly, & half treated with 5% PVP as adsorbent before H.S. centrif. Final pellets resuspended, & spun in d. grdt. column. | PVP treatment → small, clean pellets. Very little difference between D & H extracts, with or without PVP treatment. |
| 6 Oct. | 30 g lvs. & petioles. 0.01 M NaAc^- -HAc ⁺ buffer, pH 4, & 5% PVP, 0.01 M DIECA. L.S. clarif., 1% Triton, H.S. pel't. d. grdts. | No visible zones, no differences. D-H shown by fractionation. Maximum UV absorbance of pellet at 270 m μ . |
| 8 Oct. | 85 g lvs. Repeat 6 Oct. extraction without addition of PVP. | Single zone, specific to D extracts, relatively slowly sedimenting. |
| 15 Oct. | 30 g lvs. & petioles. 0.01 M NaPO_4 buffer, pH 7, 0.01 M DIECA. 1% Triton X-100, 0.05% 2-ME. butanol-chloroform clarif., H.S. pel't, d. gdt. Nucleic acid extract from H.S. pel't injected into <i>N. clevelandii</i> , petunia. | Possible extra zone in D extract. Symptoms turned out to be contamination caused. |
| 15 Oct. | 55 g root tissue. 0.06 M Tris-HCl buffer, pH 7.4, with 0.02% DIECA, 0.1% Na_2SO_3 . 8% butanol clarif. H.S. pel't, d. grdt. centrif. | Nothing of interest in either D or H extract by fractionation. |
| 20–22 Oct. | 20 g lvs. 0.05 M glycine HCl buffer, pH 3.6, with 0.01 M DIECA, 1% 2-ME. Incubate homogenate in cold room overnight; add Triton X-100 to 1%, H.S. pel't, d. grdt., infectivity assay of pelleted material & nucleic acid extract of same. | No zones detectable in extracts. No symptom development on assay plants. |
| 28 Oct. | 25 g lvs. 0.1 M K_2HPO_4 , pH 8 with 1% 2-ME; butanol clarif. H.S. pel't, d. grdt. infectivity assay of nucleic acid extracted from H.S. pel't by injection & manual inoculation of petunia, <i>N. clevelandii</i> . | Same sort of zones of material (ribosomes in various aggregation states?) in D and H extracts. Some symptom development, turned out to be contamination. |

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| 30 Oct. | 15 g leaf tissue. glycine PO ₄ -NaCl-SDS buffer & 1% Triton chloroform-butanol clarif., followed by phenol extraction for nucleic acid, polyacrylamide gel electrophoresis. | No band of N.A. peculiar to D extract noted. |
| 13-18 Nov. | 20 g leaf tissue. 0.05 M KPO ₄ buffer, pH 7, 0.02 M Na thioglycollate, 0.01 M DIECA, chloroform-butanol clarif.; H.S. pel't. pelleted material divided, spun on d. grdt. & incubated with 0.01 M EDTA in buffer, then spun on d. grdt. | Four or five bands present in both H & D extracts. Incubation with EDTA before centrifugation removed all but one minor peak, suggesting that the others were ribosomes in various aggregation states. |
| 18 Nov. | 35 g lvs. 0.05 M K ₂ HPO ₄ buffer, pH 8, & 1% 2-ME; chloroform clarif. Extract divided-some fractionated with ammonium sulfate & some not-before H.S. pelleting, d. grdt. run. | No zones in D or H extracts. |
| 25-27 Nov. | 28 g lvs. & petioles. 0.02 M K ₂ HPO ₄ , pH 8.0 & 15 g Al ₂ O ₃ & 0.5% 2-ME. Clarif. with hydrated calcium phosphate. H.S. pel't, acidified for further clarif., H.S. pel't; record UV absorbance spectra before further work. | UV absorbance of remaining material showed absorbance peak at 280 mμ — suggesting contents mainly protein, not nucleoprotein. |
| 27 Nov. | 15 g lvs. & petioles. 0.2 M NaAc ⁻ , 0.1 M EDTA & 0.5% 2-ME, pH 5.4. L.S. clarif., H.S. pel't; spun on d. grdt. | No zones in either D or H extract. |
| 2 Dec. | 20 g lvs. 0.01 M NaAc ⁻ -HAc ⁻ buffer, pH 5.4 & 1% 2-ME. L.S. clarif., H.S. pel't; spun on d. grdt. | Light scattering zones, did not show UV absorbance of nucleoprotein. |
| 3-5 Dec. | 20 g lvs. 0.05 M KPO ₄ , pH 7.4, & 0.01 M Na thioglycollate; L.S. clarif; add ammonium sulfate to 50% saturation; leave overnight in cold room; L., H.S. centrif., d. grdt. | No specific band in D. |
| 8-12 Dec. 16-18, 18-23 Dec. | 1-3 g young lvs. Grind in 0.1 M glycine, 0.05 M Na ₂ HPO ₄ , 0.3 M NaCl, 1% SDS. Clarify with butanol-chloroform, phenol extraction of nucleic acids, polyacrylamide gel electrophoresis. | Much better preparations than from older leaf tissue. However, no specific D component consistently observed. |

Screening and Quarantine of Cassava from East Africa*

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Introduction

THIS paper describes work carried out in our laboratory on the control of African cassava mosaic disease (CMD) in Guinea. It was planned to introduce into Guinea several varieties of cassava originating in Kenya and Tanzania, that showed partial resistance to CMD. However, before transfer to Guinea, the material had to be certified as free of detectable viruses and of CMD.

The screening work was carried out in our laboratory in Italy during 1974 and 1975. All other tests and also the multiplication of the material were carried out at the Istituto Nazionale Piante da Legno (INPL). The INPL also provided us with cuttings of susceptible Guinean cassava both with and without CMD symptoms. This material was used to check whether viruses other than the CMD agent were present in Guinean cassava and to make further investigations on CMD from both East and West Africa.

The most important sap-transmissible virus which we looked for was cassava brown streak, reportedly present in East but not West Africa (Lozano 1972). Secondly, common cassava mosaic (a potex virus) and cassava vein mosaic (a 50 nm spherical virus) were important possibilities (Costa and Kitajima 1972), although to date they have only been reported from South America.

Materials and methods

Plants A total of 248 cuttings from ten Kenyan varieties (4763-16; 5315-38; 5318-34; 5543-

156; 5547-24; 37244-E; 46106-27; 50284-33; 50583-14; 54140-10) and four Tanzanian ones (27; Kagu Kaninga; M'Suffi; Tandika) as well as several cuttings from Guinea were screened.

Maintenance of plants Plants were kept in an insect-proof and temperature-controlled glasshouse, and for part of the time, during the summer, they were also kept in an insect-proof screenhouse. In the screenhouse, plants were kept in pots or planted in steam-sterilized soil. The plant houses were periodically treated against insects and mites.

To check for the possible presence of viruses transmitted through the soil or by winged vectors, various bait-plants were planted in different parts of the screenhouse, and checked after 2 months. The plants used were *Chenopodium quinoa*, White Burley tobacco, and cucumber.

Healthy cassava plants were used to screen against viruses naturally present in the region of the laboratory. For this purpose plantings were made in July in three locations where both viruses and vectors were common. The plants were checked for virus infection in October.

Screening of cassava Screening for CMD was carried out by visual inspection for symptoms. All cuttings with symptoms were excluded.

Plants were checked for the presence of sap-transmissible viruses by grinding leaves, either from single plants or from groups of 3-5 plants, in either (a) a suspension of alumina and Celite (Lister 1959) or (b) 0.02 M phosphate buffer at pH 7 and containing 0.005 M DIECA, 0.005 M N-ethyglycollate and 0.001 M EDTA. The extracts were inoculated into the following test plants: *Chenopodium album*, *C. amaranticolor*, *C. quinoa*, *Cucumis sativus*, *Datura stramonium*, *Gomphrena globosa*, *Nicotiana clevelandii*, *N.*

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glutinosa, *N. rustica*, *N. tabacum* cv "White Burley," *Petunia hybrida*, *Vigna unguiculata*. Cuttings were tested twice, once with method (a) and once with method (b). Test plants were kept under observation for at least 30 days.

Electron microscopy (a) *Thin sectioning*. Selected samples of both healthy leaf material and that showing CMD symptoms were fixed, embedded in Epon, and thin-sectioned for electron microscopy using standard techniques. Tissues were surveyed for evidence of viruses or mycoplasma and for Rickettsia-like bodies.

In the greenhouses, white flies (*Trialeurodes vaporariorum*) and mites (*Tetranychus urticae*) infested the cassava plants despite frequent treatments with pesticides. In fact, the extensive spraying occasionally damaged the plants.

The cassava plants all remained healthy and no virus was detected on the bait plants.

Screening CMD appeared on cuttings of four varieties of Kenyan and on all varieties of Tanzanian cassava.

(b) *Negative staining* Checks were made for virus-like particles, using an improved "dip preparation" technique, followed by negative staining with uranyl acetate or potassium phosphotungstate. Where tobacco mosaic virus (TMV) was suspected, this virus was also looked for by the

more sensitive techniques of immunoelectron microscopy.

Treatment with antibiotics A total of 26 plants showing CMD symptoms were treated with tetracycline hydrochloride or chloramphenicol, at a level of 100 ppm in water. The treatments were applied by: (a) repeated leaf spray; (b) repeated soil drench; (c) washing the soil off the roots of the plants and soaking them once in antibiotics for 18 h.

Treatments (a) and (b) were made twice weekly for 8 weeks, each plant receiving 100 ml of solution each time. As controls, 13 plants were treated with tap water. Treatments (a) and (b) were repeated on plants of *Vinca rosea* previously infected with a mycoplasma, to check the effectiveness of the treatments (Davis and Whitcomb 1970; Duval 1970; Hopkins and Mortensen 1971; Sinha and Peterson 1972).

Results

Growth of the plants The plants grew rather poorly in the greenhouses and in the open air, while in the screenhouse (particularly if planted in the ground) they developed vigorously (Fig. 1).

No virus was isolated from the cassava brought from Africa except TMV which was present as a "tomato" strain in three plants from Kenya and as



Fig. 1. Cassava plants in the screenhouse in Turin (photo G. P. Gaviani).

a "vulgar" strain in one cutting from Tanzania. TMV did not induce any symptoms in cassava and was present in such a low concentration that it was not detectable by serology or electron microscopy in the original plants, but only after transmission through test plants. The strains of TMV were characterized by symptomatology on test plants. It was not known whether the TMV strains were present originally or were introduced by contamination.

Both the strains were back-inoculated to healthy cassava plants. No symptoms were induced, and TMV could be reisolated from these plants only with difficulty, and in low concentrations.

The few healthy cassava plants that were exposed to natural viruses in the fields near Turin remained healthy at the end of 3 months, though surrounding crop plants were largely infected. It seems from this small trial that cassava may be rather resistant to our native plant viruses, but a larger trial would be necessary to confirm this conclusion.

Using the thin-section electron microscopy technique, a careful search of material showing symptoms of CMD showed much pathological disorder in the tissues but there was no evidence of a pathological agent such as a virus, mycoplasma, or Rickettsia. These results are in line with those of Murant et al. (1974).

No virus was found in dip preparations of any cassava plant examined using the negative staining technique.

No cassava plants treated with the two antibiotics used showed any attenuation of the CMD symptoms in comparison with control plants treated with water. The plants of *Vinca rosea* used to monitor the treatments showed clear symptom remission, but only in the case of tetracycline applied by leaf spray.

Conclusions

Cassava plants grown in screenhouses during the summer showed a rapid and large increase in height, stem diameter, and leaf development. However, plants grown in greenhouses and, in summer, in the field did not grow well.

The occasional presence of TMV in cassava plants does not seem to be very important.

The trials on natural transmission of viruses to

cassava, done on the few plants available for this purpose, did not give definitive results.

The situation regarding the possibility that CMD may be caused by a mycoplasma or a Rickettsia is still confusing. First, CMD symptoms are quite unlike those reported for mycoplasmal or Rickettsial diseases; second, no mycoplasma-like bodies could be observed in the vascular tissue of diseased plants; and third, antibiotics applied in affected plants induced no symptom remission. However, the number of plants treated with antibiotics was limited. Furthermore, Costa and Kitajima (1972) reported failure to obtain symptom remission in cassava infested with a known mycoplasma.

As a result of this work, a number of cassava varieties introduced from East Africa by the INPL were tested by us and found virus-free to the best of our knowledge. The plants were then returned to the INPL, multiplied, and sent to Guinea.

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In Vitro Techniques in the Control of Cassava Mosaic Disease

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Introduction

CASSAVA is a vital crop in many developing countries as a food source and is assuming increasing importance as an export product (Nestel 1973). Africa grows 35% of the world's production of cassava. The average yield of about 6 t/ha could be raised six to ten times this amount. One very significant factor contributing to low yield and which handicaps rapid expansion of cassava production is cassava mosaic disease (CMD) which may reduce yield by as much as 65–95% (Hahn 1972). The presence of this disease not only reduces yields, but also inhibits the movement of germ plasm for breeding purposes.

The effective solution to the mosaic problem requires the implementation of a wide range of both standard and new approaches to crop management and development. The present report focuses attention on the role that in vitro or plant cell culture methods might assume in cassava crop improvement with particular reference to cassava mosaic disease.

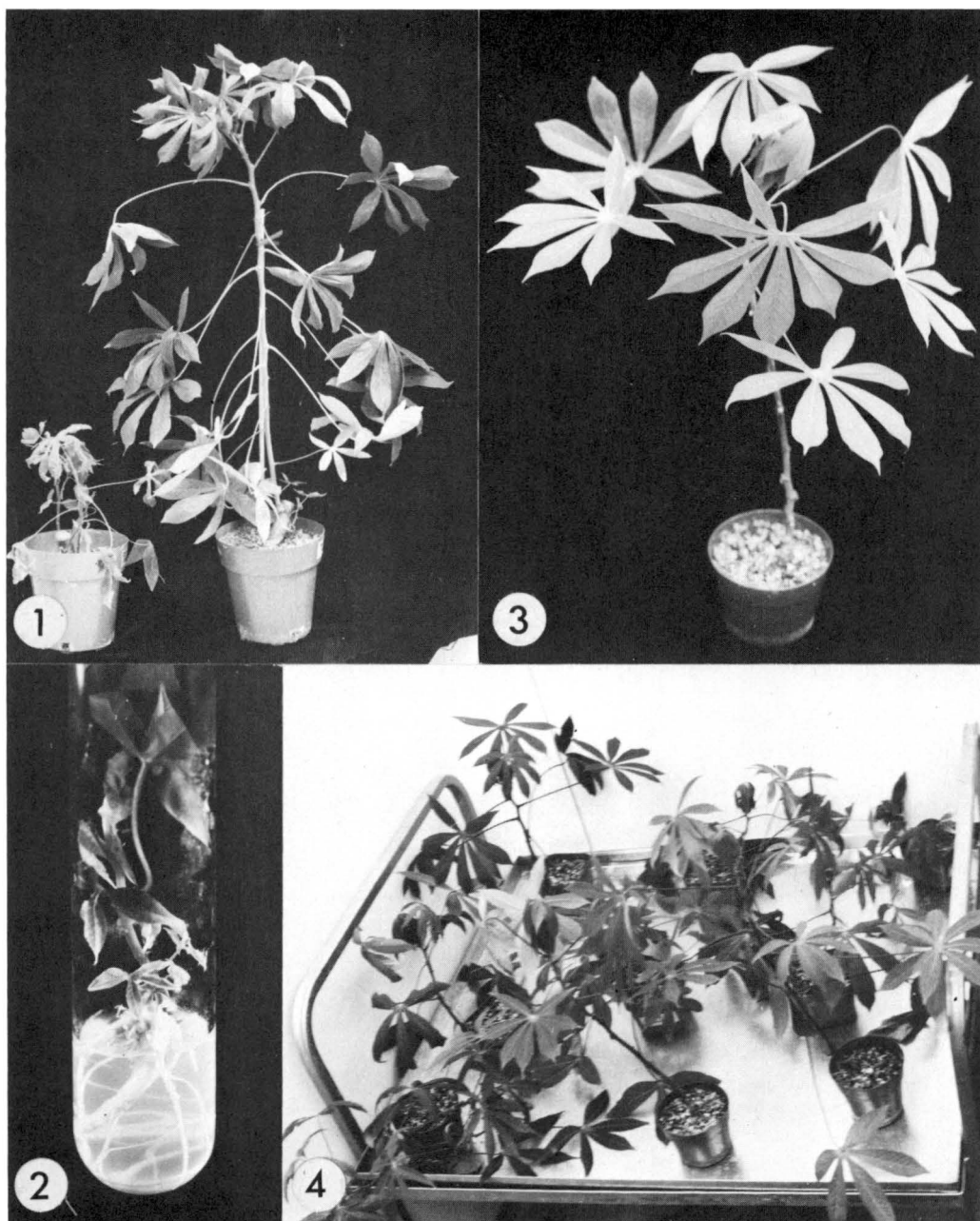
Recent advances in the development of in vitro techniques with higher plants permit their application to practical problems related to agricultural crops. Methods have become available for the growing of plant cells under controlled environmental conditions for indefinite periods. Using populations of diploid or haploid cells it is possible to carry out cloning and selection procedures analogous to those used with microorganisms. Since plants can be regenerated from cultured cells, the modifications induced in culture and expressed in the plants can be examined and the beneficial traits utilized.

Two of the immediate practical applications of tissue culture are the production of pathogen-free stock plant material by meristem culture, and the rapid propagation of new clones and cultivars by shoot tip and callus culture (Murashige 1974). Research has been undertaken in this laboratory to develop such methods for cassava. The meristem procedure that we have developed makes it possible to obtain large populations of disease-free plants (Kartha et al. 1974; Kartha and Gamborg 1975b). The results are presented and discussed below.

Elimination of the causal organism

The causal organism of CMD invades all tissues of cassava plants. The standard procedure of propagation from stem buds facilitates disease transfer from old to new plants. It has been known for some time that cells at the very tip of new shoots in plants are free of virus. These cell layers of the shoot tips can be removed, cultured in nutrient agar, and will then grow into complete plants. The method can be perfected to yield virus-free plants. This approach is used commercially for potato and several ornamental crops (Hollings 1965).

Although cells in the shoot tip region of higher plants have a high potential for division and formation of complete plants, the conditions must be ascertained for each species. Kartha et al. (1974) developed the method for regenerating complete plants from cassava which has been applied to Colombian cultivars and to infected plant material of Indian and African origin (Kartha and Gamborg 1975b).



Regeneration of mosaic disease-free cassava plant by thermotherapy coupled with meristem tip culture. 1—Cutting from diseased plant, cv. Kalikalan, grown under greenhouse conditions (*left*) and growth cabinet conditions (*right*) at 35°C for 30 days. 2—Cutting from mosaic-diseased plant, cv. Ogunjobi, grown at 35°C for 30 days. Note the disappearance of mosaic symptoms and the increased vegetative growth in 1 (*right*) and 2. 3—In vitro regeneration of cassava plantlet from the meristem tip of a diseased plant grown at 35°C. 4—Mosaic disease-free cassava plants grown from in vitro culture of meristem tips.

The procedure consists of several steps (Kantha and Gamborg 1975a, b; Fig. 1–4) (1) Sections with two nodes cut from diseased stakes were planted in pots containing vermiculite and watered by nutrient solution; (2) The stakes were incubated at 26°C for 18 h at 70% RH to permit sprouting; (3) Sections of 0.2–0.5 mm were dissected aseptically from the shoot apex of the new sprouts under a microscope in a laminar flow cabinet; (4) The sections were placed on agar nutrient medium supplemented with hormones (benzyladenine, naphthalenacetic acid, gibberellic acid) in tubes and incubated at 26°C for 16 h at 70% RH; (5) Complete plantlets regenerated within 30 days and were transferred to pots and grown to mature plants; (6) Visual observations and diagnostic procedures were performed over a 6-month period. The diagnostic methods included transmission experiments by grafting scions from regenerated plants into stocks of healthy, susceptible cultivars and using appropriate controls.

Results

Data with cv Kalikalan (Indian cultivar provided by Dr M. R. Menon): From 150 cultured meristems, 135 plants were obtained. Of these 70 were symptom-free.

Data with cv Ogunjobi (Nigerian cultivar from Dr S. K. Hahn): From 45 meristems, a total of 42 plants were obtained and 40 of these were symptom-free.

Data employing high temperature: Further experiments were performed with meristems taken from sprouts of stakes incubated at 35°C for 30 days. Tip sections of 0.5–0.8 mm were used. Kalikalan — 50 meristems, 45 plants (all symptom-free), Ogunjobi — 50 meristems, 46 plants (all symptom-free).

Plant regeneration is highly reproducible and occurs at 90% efficiency. The percentage of symptom-free plants is 90–100.

These procedures were effective in yielding symptom-free plants. The plants also are free of any virulent or transmissible causal agent.

One or more types of virus have been implicated, but information on the causal agent(s) is sparse (Beck 1971). Until the causal organism can be fully identified, the elimination of the disease agent from a stock cannot be satisfactorily verified (Appendix A).

Liu (1975) at the Taiwan Sugar Research Institute reported recently on the success of using nearly identical procedures for regenerating plants from cassava cultivars grown in Taiwan. Reports have also appeared recently on similar although

less successful methods to eliminate viruses in sweet potato, taro, cocoyam, and bananas (Alconero et al. 1975; Hartman 1974; Berg and Bustamante 1974).

Morphogenesis and propagation of healthy stock

The very high efficiency of plant regeneration from cassava meristems makes it feasible to employ the methods for the production of healthy stock (Appendix A). The further vegetative propagation of such stock can proceed by using tip cuttings and rooting hormones in humidity chambers. These chambers can be equipped with a mist system and fine gravel used as growth support (Wholey and Cock 1973).

The meristem culture operation requires relatively simple facilities and a modest working area (Appendix A). The propagation of healthy stock plants requires a larger area. Such an operation may be most economically established in a region in which vegetative growth can occur throughout the year. Other requirements are a location isolated from commercial cassava production and the absence of vectors transmitting the causal agent of mosaic. A comparable operation has been in existence for some time to produce healthy potato stock in Canada (Agriculture Canada Research Station, Vancouver, B.C.). At this station the meristem technique is used to obtain virus-free plantlets. The plantlets are then transferred to an isolated region in the interior of British Columbia where elite growers produce seed potatoes for commercial growers and for export to the United States and Holland. In tests with the healthy potato stocks, the reported yields reached 38% above that of infected plants. A similar arrangement can be envisaged for producing mosaic-free cassava stock.

Propagation by tissue culture In recent years methods have been developed for propagating plants through explants other than meristems (Murashige 1974). Procedures are employed for several horticultural crops by commercial growers. The explant material is often a shoot apex tissue. The plants obtained by this means are genetically the same. Two examples may serve to illustrate the prolific nature inherent in such procedures.

Earle and Langhans (1974) designed methods for obtaining multiple plantlets from shoot tips of chrysanthemums. These plants are normally propagated by cuttings using succulent shoots. The shoot tip technique enables one tip to produce 125 plantlets in 3 months, 15,000 in 6 months, and more than 200 million in 1 year. Dr Murashige,

who is one of the pioneers in devising these methods for commercial growers, also estimated that it should be possible to produce 300,000 plantlets of asparagus from a single shoot tip in 1 year (Hasegawa et al. 1973).

In addition to meristems and shoot tip morphogenesis, plant regeneration has also been achieved from leaf sections, e.g. tomato (Kartha et al. 1976) and rapeseed (Kartha, unpublished data). This approach has recently been applied to tobacco where plant regeneration occurred from sections of the dark green "islands" of tobacco leaves infected with TMV. Approximately half the plants were virus-free (Murakishi and Carlson 1976). Plant regeneration from callus sugar cane on a larger scale has been employed successfully to obtain new selections which are Fiji disease-resistant (Krishnamurthi and Tlaskal 1974).

Breeding resistant cultivars

The claim has been made that the most efficient, practical and economical attack on the mosaic disease problem is breeding for resistance (Beck 1971; Jennings 1972), and some success has been achieved in introducing resistance into cultivated varieties (Beck 1971; Bock and Guthrie 1976).

The germ plasm of most varieties is derived from *Manihot esculenta*, which apparently possesses no resistance. One of the wild species, *M. glaziovii*, is resistant to cassava mosaic. Crosses have been made between *esculenta* and *glaziovii* but natural barriers lower the efficiency of the process (Beck 1971) and resistance in the progeny may not persist (Hahn 1972).

The problems inherent in producing crosses between genera and species impose restrictions to crop improvement and are not limited to cassava. The transfer of desirable characteristics in plants is achieved by making sexual crosses. Incompatibility barriers of various types prevent wide crosses, which would be necessary for effectively introducing beneficial characteristics (Bates and Deyoe 1973). The most recent innovation of in vitro methods is the production of somatic hybrids between different plant genera and families (Gamborg et al. 1974). The process of hybridization involves a series of techniques in which protoplasts (cells with walls removed) are fused and on division form hybrid cells. The hybrids are isolated and recognized through the use of specific selection procedures. The final step is the growing of plants from hybrid cells.

Sufficient progress has been made to establish that fusion products from species of different plant families can form dividing cells. By fusion of protoplasts from two separate chlorophyll mutants

of tobacco, both hybrid cells and subsequent plants were obtained. The hybrids were recognized as green plants produced as a result of genetic complementation (recombination).

Advances in the technology have reached the stage where predictions can be made about its potential use in plant-breeding programs. Somatic hybridization permits expanding the genetic base far beyond what is now possible by conventional methods. It allows for new and desirable crosses and thus increases the scope for crop development. This technique holds great potential, not only for introducing genetic variability but also for making crosses which at present create problems because of lack of floral development (e.g. yams).

Another procedure with a potential practical application is pollen culture. Both anther and pollen can be cultured to yield haploid cells. In some species plants can be regenerated directly from pollen. By applying chromosome doubling procedures, the technique becomes an expedient, facile, time-saving device in plant-breeding procedures (Kasha 1975).

To date there have been no reports on research on somatic hybridization and anther culture of cassava. Most of the research in these two fields has involved cereals, legumes, and plants such as tobacco. The technique may be particularly useful in the improvement of cassava and other root and tuber crops because of the difficulties in making sexual crosses and because a somatic hybrid can be readily multiplied by vegetative propagation.

Conclusions

Cassava mosaic disease causes substantial losses in yields of cassava, particularly in Africa. In vitro techniques can be employed to supplement conventional procedures for combatting disease problems. A new and efficient meristem culture method has been developed for obtaining plants free of cassava mosaic disease symptoms. The method can be utilized in the production of disease-free planting stock. Other tissue culture methods, such as morphogenesis and somatic cell genetic techniques hold potential in providing new and effective procedures to amplify conventional techniques in cassava crop improvement.

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Appendix A

Facilities and Equipment Required for a Unit Station to Produce Cassava Stock by Meristem Culture

A. Facilities

- (1) An area for medium preparation, cleaning of glassware and storage facilities
- (2) In the same or preferably in an adjacent area, space for a transfer cabinet and stereomicroscope
- (3) Area for environmental growth cabinets and rooms
- (4) An area for potting and growing plants

B. Equipment

- (1) Laminar flow sterile transfer cabinet (available in several sizes and produced by a number of companies)
- (2) Stereomicroscope
- (3) Equipment for preparing sterile media
 - a) autoclave

- b) filter sterilizing units
- c) sterilizing oven
- (4) Growth cabinets. The number and size depends on the operation
Walk-in growth rooms, e.g. 10×14 ft (3×4.2 m) may be the most useful for meristem culture and plantlet formation.
- (5) Small equipment – test tubes, flasks, knives and other dissecting instruments
- (6) Refrigerator and freezer
- (7) Pots and growing materials (vermiculite)
- (8) Chemicals required for media presentation.

Symptomatology of Cassava Mosaic Disease and a Proposal for Further Study to Categorize the Variants*

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Introduction

VARIOUS differences in the symptomatology of the cassava mosaic disease complex have been observed in many African countries. Storey and Nichols (1938) believed that these variations were due to differences in strains of the causative agent. They categorized the strains as follows: (a) A severe strain, which induced severe leaf chlorosis. Chlorotic areas are usually large and uniformly distributed but sometimes localized; (b) A mild strain, which induced slight chlorosis, with the affected areas slightly yellower than normal. The chlorotic areas were small, and either generally distributed or localized. In this category some infected plants can be symptomless.

Dubern (1972) reported the following symptoms on the susceptible cassava variety Agba Baoule: mosaic, deformation, and stunted growth reminiscent of the symptoms of "witches" broom. A pathogenic agent was transmitted from this variety to different herbaceous plants, and in one of these, *Capsicum annum*, mycoplasma-like particles were observed. Terry (unpublished data) recorded the occurrence of an unusual "bushy dwarf" symptom on cassava seedlings of open pollinated material at IITA, Ibadan (58308). The manifestation is characterized by an abnormally large number of branches and leaves, and short internodes and abnormally small leaves with mild mosaic. Electron microscopic studies of tissues from these "bushy dwarf" plants are in progress. Terry and

Jennings (unpublished data) also recorded at IITA, the occurrence of an unusual "veinal chlorosis," characterized by chlorotic "leaf blotch" limited to the veins and accompanied by a yellowish vein netting. This symptom is similar to that described as "veinal mosaic" in Brazil (Costa and Kitajima 1972).

Both the "bushy dwarf" and the "veinal chlorosis" were observed on plants on which the typical cassava mosaic symptoms were absent or mild. The occurrence of these manifestations therefore raises the following questions:

(1) Are they caused by an agent or agents distinct from the cassava mosaic agent?

(2) Are they caused by a component of cassava mosaic disease complex to which these plants were less resistant? or

(3) Are they caused by the same agent whose effect is modified by the genetic make-up of the hosts?

In the absence of any techniques for investigating and differentiating the causal agent(s) of these symptoms, cassava mosaic investigators presently rely only on symptomatology. Nevertheless, they do recognize the possibility that strains of the same agent or different group of agents which are indistinguishable by symptomatology may be involved in what is now loosely described as cassava mosaic disease.

It is proposed therefore that a coordinated investigation of these phenomena be initiated in appropriate locations with collaborators who have a primary interest in cassava mosaic disease research.

* This paper was tabled at the meeting; the authors were unable to attend.

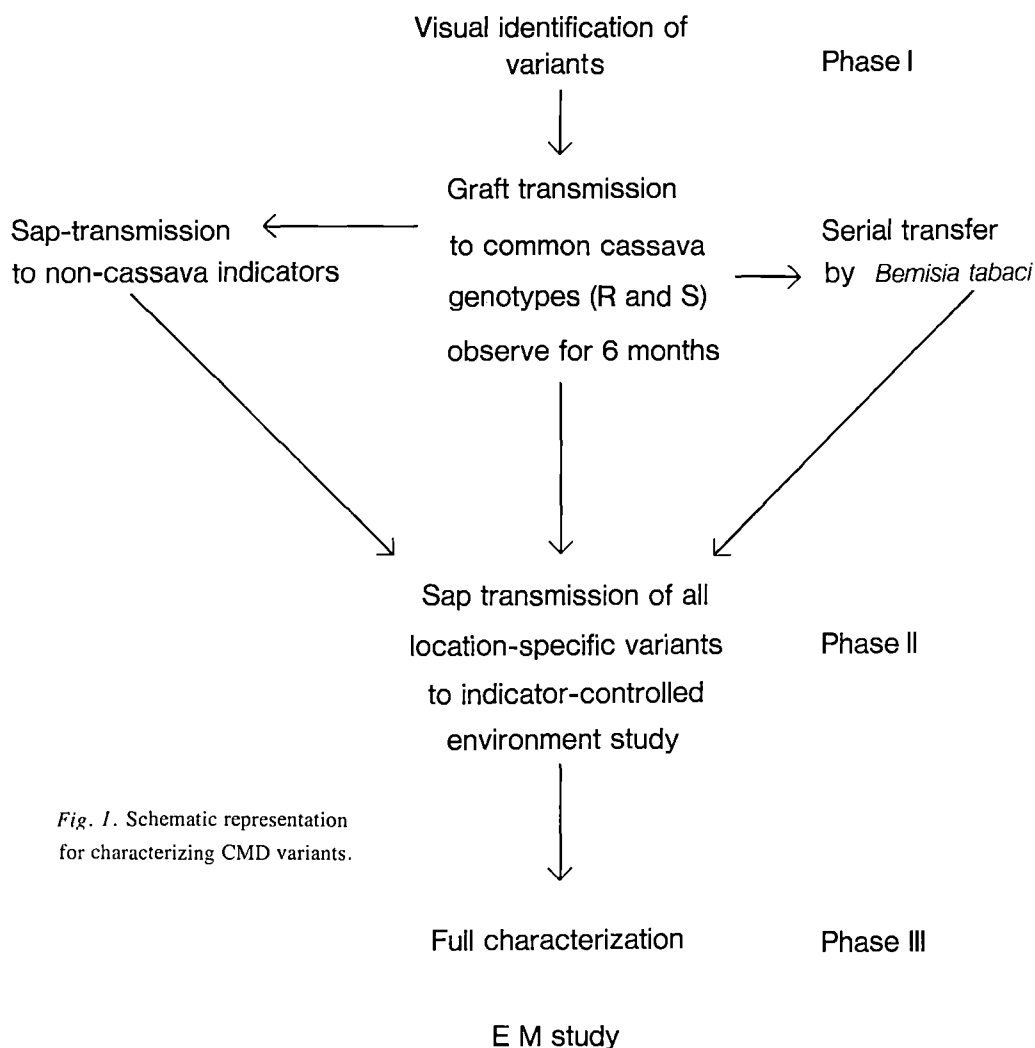


Fig. 1. Schematic representation for characterizing CMD variants.

Proposed procedure for investigations

Phase I—Location-specific 1. Visual identification of symptoms and categorization of variants. 2. Transmission of agents by grafting to two or more cassava varieties which preferably should be agreed upon by investigators in different locations. One of these varieties should be resistant and one susceptible to CMD. Successful grafting should be followed by at least 6 months of observations for stability of symptom expression. 3. Sap transmission of the agents to indicator plants other than cassava. 4. Serial transfer by *Bemisia tabaci* to hosts of differing resistance in order to separate components of the agent.

Phase II—Location-nonspecific 1. When all the different agents in a specific location have been

grafted to cassava varieties and stability of symptom expression has been achieved, the symptomatology of all the agents should be studied under controlled environmental conditions in a second location to eliminate any effects of environmental factors on symptom expression. This step will also facilitate the observation of all possible variants from different locations at a common non cassava-growing location. 2. When any or all of the different agents in a specific location have been sap-transmitted, this procedure should be repeated with all of the location-specific sap-transmissible agents at a second location under controlled environmental conditions.

It is proposed that the nonspecific location should be outside of Africa and Latin America.

Phase III—Location-nonspecific 1. Full characterization of all agents at the second or a third location.

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Breeding for Resistance to African Cassava Mosaic Disease: Progress and Prospects*

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Introduction

GERM PLASM derived from the former East African breeding program at Amani, Tanzania, is still the main source of resistance to African cassava mosaic disease (CMD) being used in breeding, and so it is useful to begin by restating the essential features of this program. It began in 1935 when H. H. Storey made a world-wide search for cultivars of *M. esculenta* resistant to CMD. He found some with a degree of resistance (e.g. F279 from Java which is still favoured around Mombasa) and obtained higher resistance (e.g. 37244E) by intercrossing some of them. However, in general he concluded that even higher levels of resistance were needed and began to transfer resistance from other species of *Manihot* by interspecific crossing and repeated backcrossing to cassava. He used the following species: *Tree species*: *Manihot glaziovii* Muell.-Arg. (Ceara rubber), *Manihot dichotoma* Ule. (Jaquie Manicoba Rubber), *Manihot catingea* Ule., 'Tree' cassava – believed to be a natural hybrid of *M. glaziovii* and cassava; *Herbaceous species*: (taxonomy disputed by Rogers and Appan, 1970) *Manihot melanobasis* Muell.-Arg. and *Manihot saxicola* Lang.

All the tree species were graft susceptible to CMD, but they conferred to their progenies a form of field resistance in which plants tended to remain free of disease or produced only mild and frequently transient symptoms. They were also resistant to cassava brown streak (CBS). Of the herbaceous species, *M. melanobasis* contributed strong resistance to CBS but not to CMD, and *M.*

saxicola was susceptible to both diseases (Nichols 1947).

Considerable progress had been made by the time that I became responsible for the program in 1951. My general conclusions on the value of the germ plasm were as follows: (1) some of the 3rd backcross derivatives of *M. glaziovii* (e.g. 46106/27) provided the best combinations of yield, root quality, and CMD and CBS resistances, but though CMD resistance was satisfactory for inland areas it was frequently inadequate for coastal regions; (2) some of the 'tree' cassava selections also showed promise, but the good resistance of the corresponding *M. dichotoma* series was not successfully combined with good yield potential. Work with *M. catingae* began late and little backcross material was assessed; (3) *M. melanobasis* was the best source of resistance to CBS, but its hybrids had a characteristic growth cycle which seemed to reduce the effectiveness of the mechanisms of resistance to both CMD and CBS, though it enhanced their yield potential (Jennings 1957, 1959).

The inheritance of resistance in the backcross progenies was assessed by the Gilbert (1967) method, which estimates the additive parental effect or 'parental value' of each parent and then calculates the statistical significance of these values and that of any interaction between them by an analysis of variance. The additive and interaction mean squares denote generalized specific combining ability respectively (GCA & SCA), and the sum of the two parental values estimates the mean expected for their progeny in the absence of specific combining ability. The results (Table 1) show that resistance to both CMD and CBS was inherited in a predominantly additive

* This paper was tabled at the meeting; the author was unable to attend.

way, though there was also a significant SCA effect for CMD resistance. A high parental value indicates a potent source of resistance: a *M. dichotoma* source had the highest, but it is interesting that only the *M. glaziovii* source is being exploited at present, resistance breeding being based almost entirely on one derivative of this species.

In view of the limited effectiveness of *M. glaziovii* derivatives in coastal regions, I attempted in 1953 to obtain higher resistance by intercrossing resistant hybrids, hoping to release recessive genes for resistance and to recombine genes which had been dispersed during the process of backcrossing. This proved successful and segregates (e.g. 5318/34) showing much higher resistance were obtained (Fig. 1). Moreover, their resistance was stable over a wide range of conditions. From this material I distributed pollinated seed to a large number of African countries in 1956. Further breeding based upon it has been reported from Ghana (Doku 1969), but the most important work occurred at Moor Plantation, Nigeria, where in 1958 M. J. Ekandem selected hybrid 58308 from seed derived from my selection No. 5318/34: this later became the main source of resistance used at IITA.

Techniques of field experimentation

In the Amani work, all susceptible genotypes planted in October–November rapidly became diseased, but plants of resistant genotypes remained symptom-free for longer, some 10–20% of them becoming diseased in April or May. For these genotypes, symptom intensity tended to be low before April–May, relatively high during these months, and then low again. Symptoms often disappeared by July, and if plants were retained after their dormant period they were normally symptom-free. It was found that a check to growth during April or May, for example by cutting the stem tips, increased the percentage of plants showing symptoms, and that this technique could be used to facilitate selection for high levels of resistance: genotypes (e.g. 5318/34) which withstood this treatment without producing symptoms also remained symptom-free when grown in the coastal environments associated with resistance breakdown. In a similar experiment done at IITA in 1975, growth checks were applied from June onwards to April-planted material of 58308. A more complicated situation was found because the younger plants had a greater capacity to recover from their symptoms. Nevertheless, the treatments considerably influence the disease intensity. In this experiment almost 100% of the

58308 plants showed symptoms at some stage, emphasizing that we are dealing with a tolerance situation and not resistance to infection. However, the conclusion is that we have a means of regulating the intensity of the disease in both the East African and IITA situations, providing an option for increasing the intensity of selection for resistance (Jennings 1957, 1960).

The present position in East Africa

The Amani program was terminated in 1957 and a collection of the germ plasm was established first at Serere, Uganda, then at Kakamega, Kenya, and finally at Mtwapa, near Mombasa. It includes the following:

| Type of Material | No. of Genotypes |
|--|------------------|
| Cultivars of <i>M. esculenta</i> | 12 |
| Backcross hybrids of <i>M. glaziovii</i> | 47 |
| Backcross hybrids of <i>M. dichotoma</i> | 7 |
| Backcross hybrids of 'Tree' cassava | 7 |
| Backcross hybrids of <i>M. melanobasis</i> | 3 |
| 3rd bc <i>M. glaz.</i> × 3rd bc <i>M. dich.</i> | 4 |
| 3rd bc <i>M. glaz.</i> × 1st bc <i>M. melan.</i> | 8 |
| 3rd bc <i>M. dich.</i> × 1st bc <i>M. melan.</i> | 3 |

All this material has some resistance to CMD, but less than 20% of the clones belong to the highly resistant category obtained by inbreeding and typified by 5318/34 and 58308. Seed material collected from the plot would therefore be expected to segregate widely for resistance and may not include representatives of this resistance category.

Five genotypes have been chosen for distribution to farmers. The choice made emphasizes the point that high resistance to CMD is not an important criterion determining a farmer's choice: none of the highly resistant genotypes was chosen.

Their first choice is 46106/27, a 3rd backcross *M. glaziovii* derivative of moderate mosaic resistance. Its popularity is based upon its root quality, reliability on sandy soil, and good habit. As well as being first choice at Mombasa, about 500 ha are grown around Tanga, Tanzania, and a smaller area near Dar es Salaam. It is largely mosaic-free on a Mombasa estate where it is grown in a large block, but is 100% diseased in farmers' plots where it is surrounded by diseased plants of local varieties. Second choice is F279, one of H. H. Storey's original importations from Java. It owes its position as second favourite entirely to the quality of its roots; 54140/10 (from intercrossing *M. glaziovii* and *M. dichotoma* derivatives), 59284/33 (3rd backcross *M. glaziovii* hybrid) and 5543/106 (from intercrossing *M. melanobasis* and *M. glaziovii* derivatives) have also been released.

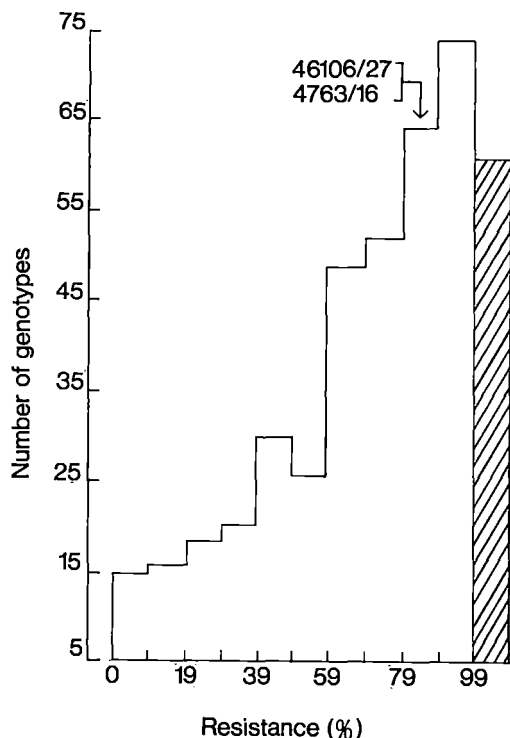


Fig. 1. Segregation for CMD resistance following intercrossing of 3rd bc *M. glaziovii* hybrids (resistance level of parents indicated by arrow).

because of their high yield potential, but they are recommended only for livestock feeding because of their high HCN content (above 50 mg/kg fresh root).

Breeding in West Africa

Hybrid 58308, derived from Amani germ plasm,

is the main source of resistance being used by S. K. Hahn (1972, 1973, 1974), at IITA. It has contributed improved resistance to the IITA populations, but resistance tends to be recessive, and selections from the first generation of crossing are rarely as resistant as this parent. Nevertheless the symptoms are usually only moderately severe and transient, and yield reduction due to CMD infection is consequently minimal. Big advances in agronomic merit are being obtained by using breeding material from Latin America. This is contributing genetic factors which affect yield directly, and it is considered inadvisable to select too vigorously for CMD resistance amongst types with such good yield potential. The breeding method allows for intercrossing among large populations of improved plants, and so recessive CMD resistant forms like 53808 segregate in each generation (Fig. 2). Nevertheless, in a situation where big advances are possible by selecting directly for yield, and where the level of resistance is such that CMD has minimal effect, it seems unlikely that the resistance level of 58308 will be achieved by a high proportion of the selections, at least in the early stages of breeding.

It is undoubtedly correct to exploit this germ plasm even at the expense of high CMD resistance, which can be restored by further breeding. At this stage it is difficult to envisage situations where higher levels of resistance may be needed, but various possibilities could be discussed. For example, although the consequences for yield of low-severity CMD have been found negligible in the germ plasm studied so far, it is possible that selection for higher yields could produce genotypes for which high resistance is a greater asset than at present; it is possible too that 58308 derivatives may be found wanting in certain environments or when attacked by certain strains of the agent, or again the introduction of new germ plasm may

TABLE 1. Additive parental values for resistances to CMD and CBS.

| Parent | Origin of parent | CMD | CBS |
|--|----------------------------|------|------|
| 37244E | Java × Madagascar | 21.7 | 7.5 |
| Msitu | Local Kenya | 10.9 | 3.4 |
| Aipin Valenea | Brazil, via Congo | 3.1 | 29.1 |
| Macaxeira aipin | Brazil | 6.0 | 25.7 |
| Mbarika | Local, Tanzania | 0.2 | 8.8 |
| 4070 | 1st bc <i>M. dichotoma</i> | 28.3 | 32.4 |
| 43101/32 | 2nd bc <i>M. dichotoma</i> | 46.2 | 4.7 |
| 4032 | 1st bc <i>M. glaziovii</i> | 32.4 | 62.0 |
| 4318/22 | 2nd bc <i>M. glaziovii</i> | 30.0 | 49.2 |
| <i>M. melanobasis</i> | Surinam | 11.6 | 82.6 |
| Percentage of between-family variation | | | |
| Parental values (G.C.A.) | | 69.6 | 88.7 |
| Interactions between parents (S.C.A.) | | 30.4 | 11.3 |

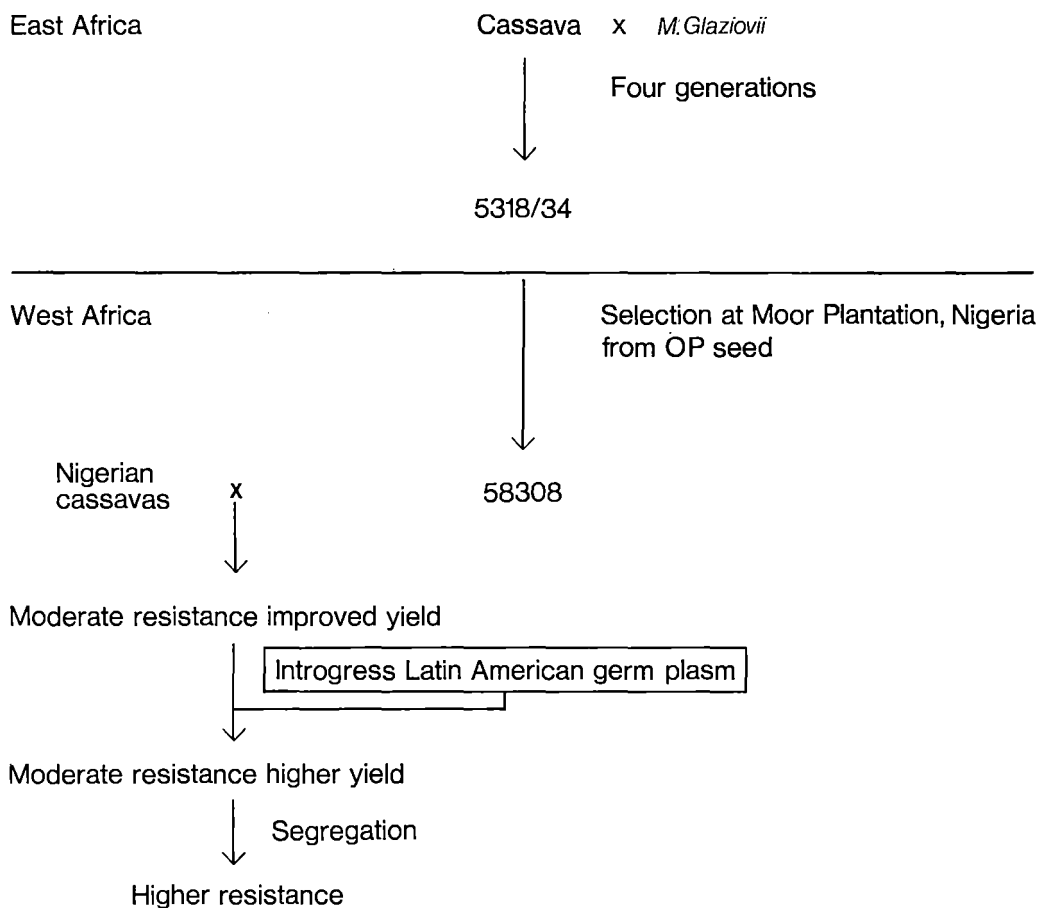


Fig. 2. Breeding for resistance to CMD at EAAFR0 in East Africa and at IITA in West Africa.

disrupt the resistance mechanism of the plants, as happened when I attempted to utilize germ plasm derived from *M. melanobasis* to improve yield (Jennings 1959, 1960). Thus, while we should maintain all our sources of resistant germ plasm so as to be able to respond to any such unforeseen circumstance, there seems no reason to question the considerable potential of the resistant material emerging from the present IITA breeding program. The parents of 58308 had adequate resistance for East Africa, the resistance of 58308 itself has been shown adequate for Nigeria and so has that of its progeny in Zaïre, Sierra Leone, and Liberia. It is likely that farmers will insist on planting mixtures of cultivars and that new cultivars will generally be grown adjacent to diseased plants and subject to continuous attack by infective vectors: a good resistance level will be required even if it is not the highest attainable.

Genetic studies in West Africa

Hahn has also used Gilbert's method to analyze his data, and for crosses among six parents he obtained the parental values given in Table 2 (Hahn 1973). He found that 58308 also contributed resistance to cassava bacterial blight (CBB), and concluded that resistance to CMD was controlled by quantitative genes with additive effects; it appeared to be a recessive character with a heritability of about 60%. The conclusions regarding CMD resistance are similar to those from the East African work.

During a recent stay at IITA I studied the inheritance of resistances to CMD and CBB in a 6×6 diallel. The results (Table 3) accord with earlier ones and further analyses by W_r/V_r regression (Jinks 1954) emphasize the strongly recessive nature of genes for resistance to each disease (Fig. 3) (the positions on the graphs of

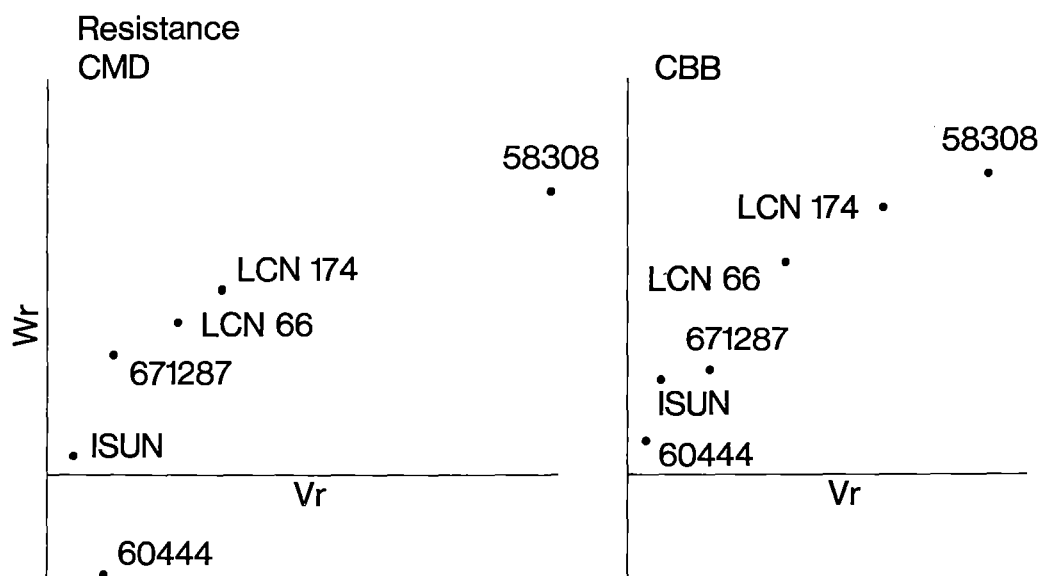


Fig. 3. Wt/Vr analyses for resistance to CMD and CBB in a 6×6 diallel recorded at IITA.

TABLE 2. Parental values for resistance scores to CMD and CBB.

| Parent | CMD ^a | CBB ^a |
|-----------------------------|------------------|------------------|
| 58308 | 1.505 | 1.105 |
| 58198 | 0.789 | 1.568 |
| Isunikakiyan | 1.667 | 1.846 |
| Oyanrugba Funfun | 1.636 | 1.758 |
| Oyanrugba Dudu | 1.641 | 1.681 |
| Ogunjobi | 1.485 | 1.672 |
| <i>Analysis of variance</i> | | |
| General combining ability | 9.558** | 8.698** |
| Special combining ability | 0.755 | 1.093 |

^a Low scores denote high resistance.

58308 indicate that this parent carries mostly recessive genes for each resistance and those of LCN 66 and LCN 174 indicate that these parents are heterozygous).

My main objective was to determine whether the resistances to CMD and CBB were genetically linked. The evidence will be presented, though further work is necessary before definite conclusions can be drawn. Two particular results are relevant here. First, in an experiment where genotypes were propagated clonally for trial, a significant within-genotype (i.e. nongenetic) correlation was obtained between the intensities of CMD and CBB ($r = 0.385$, $df = 403$). Since CMD was present before the attack by CBB, a reasonable conclusion is that the presence of CMD weakened resistance to CBB. Hence it is particularly important to have CMD resistance where CBB is a

problem. Second, linkage between the two resistances, if confirmed, would suggest a very interesting genetic situation: the two resistances must derive from *M. glaziovii* seven generations of strong selection for non *M. glaziovii* characteristics have occurred since the first interspecies cross. Linkage persisting to this stage therefore suggests that blocks of resistance genes may be held together in a chromosomal situation which restricts their recombination with genes from *M. esculenta* — a situation which calls for cytogenetic study.

Recommendations

I would like to convey two ideas in this paper: the need to exploit the progress that has been made, and the need to conserve germ plasm so as to be able to respond to any new situation that might

TABLE 3. Parental values for percent resistance to CMD and CBB.

| Parent | CMD | CBB |
|-----------------------------|--------|--------|
| 58308 | 24.5 | 32.4 |
| *LCN 66 | 16.4 | 20.9 |
| *LCN 174 | 16.4 | 26.2 |
| 671287 | 13.2 | 18.4 |
| Isunikakiyan | 6.6 | 8.5 |
| 60444 | 0.7 | 9.1 |
| <i>Analysis of variance</i> | | |
| | MS | MS |
| G.C.A. | 5611** | 7914** |
| S.C.A. | 722* | 335* |

* LCN 66 and LCN 174 are both from the cross 58308 \times Isunikakiyan.

arise. I need hardly say that control of CMD is unlikely to be achieved by any means other than by the use of resistant cultivars, and therefore suggest that the highest priority should be given to these two needs. My recommendations are: (1) Exploit IITA germ plasm by selecting for local adaptation at as many centres as possible in Africa and make appropriate arrangements to propagate rapidly and disseminate the selections. Some of the Mtwapa clones may also qualify for this. (2) Maintain seeds representing all sources of CMD resistance so that they are available for any contingency which might arise in the future. The facilities of the new germ plasm conservation unit at IITA should be used. (3) Arrange for seed from the Mtwapa collection to be moved to IITA for introgression into the breeding populations there. (Note that item 2 is suggested in addition to item 3, since new sources of resistance may be lost in a situation where selection is for *adequate* resistance combined with high yield potential). Available *M. dichotoma* derivatives for example are poor yielders. The alternative of creating a mosaic-resistance composite where first priority in selection is for CMD resistance can be postponed until a need is shown to exist. (4) Pursue genetic studies of CMD resistance, including resistance from sources not yet studied genetically,

and also cytogenetic studies of 58308 and the association between resistances to CBB and CMD.

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Summary of General Discussion

In opening the discussion *Nestel* suggested that a suitable take-off point might be to consider the progress that had been made and that which still needed to be made in terms of the research activities proposed at the IITA Workshop (page 44 of IITA meeting report). As a result of this suggestion the discussion focused on five issues.

Characterization of the causal agent(s)

It had been suggested at the IITA Workshop that characterization might be facilitated by first transmitting the agent to a Solanaceous host such as cucumber, however none of the participants in the workshop had been able to repeat the claim of Indian workers that cucumber was an alternate host. Both *Luisoni* and *Peterson* tabulated a large number of potential alternate hosts to which they had unsuccessfully tried to transmit African mosaic virus. They had had some success, and *Bock* and *Guthrie* had had even further success, with *Nicotiana clevelandii*. Using this species *Bock* and *Guthrie* were able to obtain different symptoms with brown streak and the presumed mosaic virus. However, they had not yet been able to transmit either virus back from *N. clevelandii* into *M. esculenta* nor had they developed an effective anti-serum for the brown streak virus.

The EAAFRO workers had been working on African cassava mosaic for more than 20 years and it is possible that during this period changes in the varieties of cassava used may have resulted in their being less susceptible to brown streak.

Thus the absence of brown streak symptoms, other than on the coast of East Africa, may be related to varietal tolerance in cassava. *Peterson* commented on the latex artifacts that could be readily confused with virus particles in leaf impressions using West African material, and suggested that his work on this warranted repetition using an isolate of the brown streak virus. (This comment may be related to an earlier claim by other workers that a rod-shaped virus was associated with the disease—Ed.) Further work appeared to be necessary on the purification of the mosaic virus and on serological comparisons of strains from different parts of the world. It was suggested that workers in a non-tropical cassava-free location should acquire susceptible plants from East Africa, West Africa, and India and, after cleaning these plants by the tissue culture technique, should conduct cross-grafting experiments in order to assess whether different viral strains existed. *Terry* and *Jennings* provided a detailed work plan for implementing this suggestion.

Transmission studies

Closely associated with the need for further work on characterization was the need for additional transmission studies using vectors, tissue fusion, and mechanical methods. Considerable discussion took place regarding the series of field plots visited at the coast in which clean material of both disease-prone and tolerant cassava lines was grown in various isolation blocks. It appeared that by the simple cultural practice of planting clean material away from diseased stock, the incidence of mosaic could be reduced to 6% or less. Even in

circumstances when alternate rows of clean and infected mosaic were planted side by side the transmissibility between the rows appeared to be low. The preliminary results from a comparative yield trial harvested by the workshop participants indicated that the reduction in yield was of the order of 70% in a tolerant line but rose to 86% in a susceptible variety. The low spread of infection between the rows led the Kenya participants to suggest that the greatest factor in the spread of infection was man.

Tissue Culture

In order to assess the value of the meristem tissue culture technique developed by *Gamborg* and *Kartha* it was recommended that this material should be evaluated in field trials alongside cassava of the same variety that had passed through four or more disease-free generations in the greenhouse, since evidence existed that the disease could remain latent throughout at least two generations of plant growth. It was suggested that the tissue culture technique might be of considerable importance in the production of virus-free stocks although further work was needed to fully evaluate whether “symptom”-free material produced by tissue culture was in fact “disease”-free. The participants noted with considerable interest the proposal of the Saskatoon workers to examine the possibility of preserving cassava germ plasm through a combination of liquid nitrogen storage and meristem culture and considered that such an approach could be of great value in terms of the production of mosaic-free material.

Epidemiological studies

The next theme of the discussion covered *epidemiological studies* and specifically the study of the biology and behaviour of the insect vector since it was felt that this might lead to the development of field control methods other than host plant resistance. There appeared to be a need to develop techniques for culturing the white fly in captivity and for studying field movements and cycles of *B. tabaci* in both East and West Africa. *Clearwater* pointed out that little is known about either the breeding or feeding habits of the fly or about the extent to which it is resident in cassava plants. The full significance of the white fly as a vector of mosaic is also not understood nor is there any knowledge about its feeding habits on alternate hosts or whether other insects are involved in the transmission of cassava mosaic. A further factor whose role in disease transmission is not understood is the relationship between leaf cyanide level and white fly feeding habits.

Field resistance and field control

The discussion on various methods of field control and on the development and study of field resistance was somewhat limited by the absence of both *Jennings* and *Terry*. This was particularly so in the case of field resistance since the discussion on this theme was to have been led by *Jennings* who was responsible for important aspects of the EAAFRO breeding work in the 1950s and had subsequently discovered that field resistance at IITA was related to the importation of EAAFRO material in 1958. This observation is of some interest since the material concerned was a backcross with *M. glaziovii*, and it appears that the best field resistance encountered in India may be associated with importations of Madagascar cassava scions which may also be derived from *M. glaziovii* crosses. The situation is, however, complicated by the fact that the tree cassava, commonly identified as *M. glaziovii* in other parts of the world, has in East Africa been classified by the Kew Herbarium as *M. esculenta*. However, *Grant* of McGill University, who has studied the chemotaxonomic classification of cassava cultivars based on their phenolic constituents, claims that the East African “tree cassava” is likely to be a *glaziovii*–*esculenta* cross.

During the discussion on field control both *Wilson* and *Seif* stressed the importance of economic issues and the very low adoption rate for known technology for field control in Kenya, given the absence of a strong and stable market for cassava. During the field visit, it was suggested that there was a need for work on field control, supported by the development of a small-scale cassava chipping industry. This combination could provide a sufficiently strong market thereby encouraging farmers to grow cassava as a cash crop as well as a famine reserve. In the absence of such an incentive, and until land pressure becomes more acute in the cassava-growing areas of Kenya, there appears little likelihood that farmers will take the trouble to ensure that planting material is clean and derived from tolerant cassava lines.

The discussion on field control indicated very clearly that cultural practices were preferable to insecticides as a technique for vector control, and the evidence produced by *Bock* and his co-workers indicated strong support for this attitude.

The meeting identified a major need for more information on baseline data relating to cassava production in Africa, and for better measurements of the magnitude of the losses attributable to mosaic. EAAFRo recently decided to produce an information booklet relating to cassava, however the participants felt that it was necessary to go beyond this. A strong case could be made for an in-depth review of the 40 years work on cassava mosaic conducted by EAAFRo and the national research institutions which both predated it and worked alongside it. More information is needed concerning cassava mosaic research in Madagascar associated with the programs of *Cours* and of *Arraudeau*.

At the present time there are no clear indications whether field resistance is resistance to the causal agent or to the vector. There is a need to replicate test material in various locations to expose it to a range of environments and possible strains of the causative agent. In this context, reference was made to the two recent IDRC-sponsored workshops dealing with the interchange of cassava germ plasm as offering potential guidelines for the safer interchange of both seed and vegetative material. Because of the difficulties involved in interchanging plant germ plasm between East and West Africa and India, in all of which locations CMD is believed to occur, the need for collaborative programs with laboratories in non-cassava-producing countries was strongly supported. In view of the importance of the disease, there appeared to be very limited resources being devoted to research on it and most of these resources were working in isolation. On the other hand, if the degree of field control being achieved experimentally in Kenya could be repeated elsewhere, it was clear that major reductions in the losses attributable to mosaic might be brought about at limited cost. Nevertheless the achievement of this goal appeared to be closely linked to economic factors, so that a clear definition of research priorities might be difficult until more bench-mark agro-economic data are available.

Given this complex situation the workshop indicated that the following priorities could be identified as justifying immediate action:

- 1) *The development of closer working linkages among scientists investigating different aspects of CMD.*
- 2) *The need for comparative examinations of both clean and mosaic-infected cassava derived from different locations in Africa and India, to examine the degree of variation in the behaviour of the virus(es) and the degree of tolerance to the disease in resistant material.*
- 3) *The evaluation in other mosaic-affected areas of the techniques of field control developed in Kenya and the incorporation of the Saskatoon technique of meristem culture into programs for producing clean material for field control.*
- 4) *An intensive study of the biology and behaviour of Bemisia tabaci to determine the exact role of this and, perhaps, other insects in the transmission of African Cassava Mosaic.*

Other Cassava Publications

- Report of a CIAT/IDRC-sponsored cassava program review, Cali, January 1972; published by CIAT, Apartado Aéreo 67-13, Cali, Colombia.
- Report on an IITA/IDRC-sponsored workshop on cassava mosaic, Ibadan, December 1972; published by IITA, Ibadan, Nigeria.
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